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PHYSIOLOGICAL CHEMISTRY.

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——PREPARED BY——

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PREFACE.

Every laboratory has its individuality and every teacher in chemistry must adapt another's text to his peculiar circumstances or make one of his own. The American School of Osteopathy is unique and hence no other school has a text-book altogether suitable to its use. This fact is the excuse for printing another text-book in chemistry. The author is conscious of shortcomings in this brief work, there are probably more which he has not noted. He hopes by use to be able to improve later editions, until it shall contain in concise form the knowledge of the chemistry of the human body necessary to make an intelligent student of those who complete the course herein given. I have consulted the works of Novy, Halliburton, Lea, Hammarsten, Long, Gamgee, Sterling and others, besides periodical literature on the subject. Having rarely had access to original papers I have been unable to give credit to the proper authorities in many cases.

C. W. Proctor.

Physiological Chemistry.

INTRODUCTION.

The human body is composed of a number of elements united into more or less complicated compounds. The composition of living tissue can be inferred only from the analysis of the dead tissue; and many of the changes going on in a living body are very difficult to ascertain. There are, however, many changes that are known with reasonable certainty, and a study of these, together with compounds excreted, is of great interest and importance.

The elements found in the body are, according to Kirk, carbon, nitrogen, hydrogen, oxygen, sulfur, phosphorus, fluorin, chlorin, iodin, silver, sodium, potassium, calcium, magnesium, lithium and iron, and occasionally, traces of manganese, copper and lead. These are for the most part in compounds; though oxygen and nitrogen are found in the blood, and hydrogen in the alimentary canal.

The simplest classification of the organic chemical compounds found in the human body is that of *carbohydrates, fats and proteids.* Some chemists use the term albuminoid as equivalent to proteid, while others restrict it to include only gelatin, chondrin, nuclein, etc.

CARBOHYDRATES.

This group has been defined as containing those compounds which have six atoms of carbon, or a multiple of six, and have H and O in the ratio in which they exist in water. There are compounds undoubtedly belonging to the carbohydrates which have from 4 to 9 atoms of carbon, and others—as rhamnose, $C_6H_{12}O_5$—which have H and O in a ratio different from that in water.

The starches, sugars and gums, besides some allied compounds, compose this group. They are found chiefly in vegetable tissue, but some exist in the human body. Those most abundant in animal tissue are glucose, glycogen, lactose and maltose. Carbohydrates may be classified as follows:

1. Monosacharids or Glucoses.
   Hexoses ($C_6H_{12}O_6$), Dextrose, levulose, galactose.
   Pentoses ($C_5H_{10}O_5$).
   Rhamnose ($C_6H_{12}O_5$).
2. Disaccharids, or Saccharoses ($C_{12}H_{22}O_{11}$).
   Cane sugar, milk sugar, maltose and isomaltose.

3. Polysaccharids, $(C_6H_{10}O_5)n$:

Starch, glycogen, dextrin, cellulose and gums. The derivatives of starch amylo-dextrin, erythro-dextrin, and achroo-dextrin are also included in this group.

Monosaccharids.

Pentoses ($C_5H_{10}O_5$).—A pentose has been obtained from the decomposition of a glyco-proteid obtained from the pancreas. It has been found recently in several urines. Pentoses are reducing agents, but do not ferment with yeast.

Hexoses ($C_6H_{12}O_6$):—*Dextrose or grape sugar* is found in honey, fruits and in many plants. It also exists in small quantities in the blood (0.1 per cent—0.2 per cent), in urine and in various organs and tissues of the body. It is found in large quantities in the blood and urine in diabetes. It occurs in the urine after the administration of hydrocyanic acid, phloridzin, uranium salts, in CO poisoning, and when the supply of oxygen is insufficient. Dextrose is formed from pancreatic digestion. After digestion of large quantities of cane sugar, lactose or glucose, a reducing substance appears in the urine; this condition is called "alimentary glycosuria."

Dextrose and levulose may be prepared by heating cane sugar with dilute mineral acids, (inversion). It is also formed by placing a quantity of yeast with cane sugar and putting in a warm place for a few hours; after the cane sugar is broken up into dextrose and levulose, these two will ferment and form alcohol and carbon di-oxide.

Starch heated with dilute mineral acids yields first dextrin, finally dextrose.

Dextrose can be obtained in minute crystals, anhydrous, or with one molecule of water. Its crystals are six-sided plates from concentrated aqueous solutions, and monoclinic from alcohol. It is insoluble in absolute alcohol. It turns the plane of polarized light to the right. Dextrose reduces an alkalin copper solution and precipitates Cu_2O. One molecule of the sugar will reduce almost five atoms of copper. Hence by such a solution a quantitive determination may be made. The sugar forms by the oxidation of tartronic acid, $[CHOH\ (CO_2H)_2]$. The structural formula for dextrose is $CH_2OH(CHOH)_4CHO$; by this we notice that it is an aldehyde, while levulose with the formula $(CH_2OH)_2(CHOH)_3CO$ is a ketone.

Laboratory Work—Glucose:—(1) Place about 1g. dry grape sugar in a test tube and heat; note changes in condition, color and odor. Allow the test tube to cool and then add water and warm. Note the color of the liquid.

The caramel obtained by the dry heat is a harmless coloring matter, much used to give a suitable color to vinegar, liquors, etc.

(2) To about 1g. dry glucose add an equal volume of concentrated sulfuric acid and allow to stand. Note the change in color at intervals for ten minutes while engaged in further tests. Heat the acid and sugar

gradually; note the effects. The black color is due to humin substances Laevulinic acid ($C_5 H_8 O_3$) is formed at the same time.

(3) To about 2 c. c. of a 2 per cent sugar solution, add an equal volume of a strong Na OH solution and boil. Note the changes in color. The sugar undergoes oxidation in an alkaline solution. This is called the Moore-Heller test. When this reaction is applied as a test in urinalysis, a precipitate of earthly phosphates is formed and the brown color produced.

(4) To about 2 c. c. of a silver nitrate solution add ammonia, one drop at a time, until a precipitate forms and barely redissolves; add to this about 2 c. c. of the sugar solution and a few drops of NaOH and warm gently. A mirror of metallic silver forms on the inside of the tube. What kind of a process has been performed? Grape sugar will reduce a ferricyanid to ferrocyanid, indigo blue to indigo white; will decolorize litmus, etc.

(5) Trommer's Test—Render the solution strongly alkaline with NaOH or KOH, and boil, then add a few drops of copper sulfate solution and heat again—a reddish or yellowish precipitate of cuprous oxid is thrown down. When this test is tried with urine, Novy says that a white precipitate of uric acid and nuclein bases (alloxuric bodies) may form, If too much copper is added a black precipitate of cupric oxid will form.

(6) Fehling's Test—Boil some Fehling's solution in a test tube, then add one half c. c. of the sugar solution (or suspected urine) and maintain the liquid for half a minute at the boiling point. If 0. 1 per cent of sugar is present a yellow or red precipitate of cuprous oxid will appear If none appear at once add more solution to be tested. Some other constituents of urine may reduce this solution slightly. Very small amounts may fail of detection, as creatinin may prevent the precipitation of traces of cuprous oxid. It is necessary to keep the constituents of Fehling's solution separate until just before using, when equal volumes ot the constituents are mixed to form the solution.

(7) Haines' Test.—Take about 4 c.c. of Haines' solution, boil, add 6 to 8 drops of sugar solution; heat again and a precipitate of cuprous oxid will form. This solution is more stable than Fehling's. Do not boil more than half a minute after the addition of the reagent. If no precipitate forms add a few drops more of the sugar solution.

(8) To some Barfoed's solution add grape sugar solution and boil, After standing, a small amount of red cuprous oxid is precipitated. The precipitate is not so abundant and does not appear so quickly as in alkaline solutions. This reaction is important because milk sugar, cane sugar, maltose and dextrin do not reduce it. Barfoed's solution is prepared by dissolving 1 part of copper acetate in 15 parts water. To 200 c.c. of this solution add 5 c.c. of a 28 per cent solution of acetic acid.

(9) Boettger's Test.—To about 4 c.c. of the sugar solution, add an equal volume of NaOH solution, then introduce a very small amount of bismuth sub-nitrate, and boil from 2 to 3 minutes. A black precipitate,

which late investigators prove to consist mainly of lower oxids of bismuth is formed. If reaction does not occur at once, let it stand ten minutes. Albumin will cause a dark precipitate of bismuth to form; hence, in making tests of urine containing albumin, the albumin must be first removed.

(10) Nylander's Test.—To 10 c.c. of the sugar solution add 1 c.c. of Nylander's solution and boil 2 to 3 minutes. Nylander's solution is prepared by dissolving 10.33 g. NaOH in 100 c.c. of water; add 2 g. bismuth sub-nitrate and 4 g. Rochelle salts. Novy says that concentrated urines containing chrysophonic acid ($C_{15}H_{10}O_4$) give a dark color, but that the test is more delicate than Fehling's.

(11) Molisch's Test.—Add one half drop of a 15 per cent solution of alpha napthol ($C_{10}H_7OH$) to one c.c. of sugar solution. Then add slowly, so that it will glide down the side of the inclined tube, about one c.c. of concentrated sulfuric acid. The acid will remain at the bottom, if carefully introduced, and a layer of a reddish violet color will form on its surface. This reaction is due to the formation of furfurol (C_4H_3OCHO), and is given by other carbohydrates. If this test is given by urine diluted with five parts of water, carbohydrates are in excess.

(12) The Phenylhydrazine Test.—On heating with a grape sugar solution phenyl hydrazin hydrochlorid, it forms yellow crystals of definite melting point called glucosazone [$C_6H_{10}O_4(C_6H_5N_2H)_2$]. This reaction is used when doubt exists after the use of the simpler tests, or when the detection of small amounts is a matter of importance. Each sugar yields characteristic crystals whose melting points determine which sugar is present. The melting point of phenyl glucosazone is 204–205° C., its crystals are needle shaped, single, or in rosettes. *Purdy applies the test to urine as follows:* To 25 c.c. of suspected urine add 1g. of phenyl hydrazin hydrochlorid, 0.75g. sodium acetate, and 10 c.c. distilled water. Place in small beaker and warm over water bath for at least an hour; remove and allow to cool. The yellowish deposit will show the characteristic needle-like crystals if the least sugar be present. It gives no reaction with the other constituents of urine.

(13) Fermentation Test.—Fill a test tube in which a small lump of yeast has been mixed with solution to be tested. Insert a stopper containing an ordinary piece of glass tubing, bent into U shape. Invert the tube and place in a beaker. If the stopper is under water the escape of gas is prevented. Allow to stand in a warm place over night. The fermentation will form CO_2 gas in the tube which is an approximate measure of the quantity of sugar. Cane sugar ferments less rapidly. If an equal quantity (100 c.c.) of urine is placed in each of two bottles with holes in the stoppers for the escape of gas, and a lump of yeast dropped into one, so that fermentation will occur in that one and not in the other; the loss in specific gravity after fermentation has ceased (in about 24 hours) will be a measure of the amount of sugar present. The degrees of density lost equal the grains of sugar per fluid ounce. This method was suggested by Roberts.

Levulose—$CH_2OH.CO.(CHOH)_3CH_2OH$.

This sugar is a ketone and occurs in many fruits and in honey. It has been found in small quantities in blood, urine and muscle. When administered in diabetes a part is eliminated as such, while part is changed to glycogen or glucose. It is supposed to be less injurious in diabetes than other sugar. Levulose is prepared from heating cane sugar with mineral acids. This is called "inversion" because the levo-rotary power of levulose is so much greater than the dextro-rotary power of cane sugar, that after the process the mixture of dextrose and levulose is levo-rotary. It is also prepared by the first action of yeast on cane sugar, and like dextrose will form alcohol and carbon di-oxide if the fermentation is continued. It responds to all the tests given for dextrose, but may be distinguished by its action on light and may be separated from dextrose by forming an insoluble compound with calcium hydroxid, while the dextrose is soluble. It forms a glucosazone identical with that of dextrose.

Galactose—($C_6H_{12}O_6$).

When milk sugar (lactose) is boiled with dilute mineral acids, dextrose and galactose are formed. It is identical with "cerebrose" which Thudichum prepared by boiling certain substances obtained from the brain, with dilute mineral acids. If may be separated from dextrose by its greater solubility in absolute alcohol, and it possesses greater specific dextro-rotary power. It ferments with yeast, but not so rapidly as dextrose. It reduces Fehling's solution. Its osazone melts at 193°. On oxidation with nitric acid it yields mucic acid ($C_6H_{10}O_8$), while dextrose yields saccharic acid, an isomer of mucic acid. Saccharic acid is very soluble in water, mucic slightly so.

Mannite—($C_6H_{14}O_6$).—This is regarded by some as a carbohydrate, but is generally considered as an alcohol.

Inosite—($C_6H_{12}O_6$).—This has a sweet taste and is crystallizable, but is regarded by very good authorities as a derivative of the benzene series, having the structural formula $(CHOH)_6$. It does not reduce Fehling's solution, has no rotary action on light and does not form osazones. It is found in muscle, especially in heart muscle, and in lung tissue. It has also been found in kidneys, spleen, liver and brain. It occurs in urine in diabetes and in Bright's disease. In the vegetable kingdom it is quite abundant in unripe beans.

Disaccharids or Saccharoses.

Cane Sugar, Sucrose,—($C_{12}H_{22}O_{11}$).

This sugar is not found in animal tissue, but it is such an important food that it is of interest to the student of physiology. Its most interesting reaction is that by which it breaks up into dextrose and levulose, either when heated with dilute mineral acids, or under the influence of invertin which is present in yeast. Its solution is strongly dextro-rotary. It crystalizes in mono-clinic crystals which melt at 160° and if heated farther yields caramel.

It does not form an osazone because it is inverted in the process and the glucosozone is formed. With sour milk, cane sugar readily undergoes lactic acid fermentation. If injected into the blood it is eliminated, unchanged, by the kidneys; it is therefore not assimilable. When taken as food into the alimentary tract it is not eliminated unchanged; it must therefore be inverted by the action of enzymes. This inversion begins in the stomach.

Maltose—($C_{12}H_{22}O_{11}+H_2O$).

The action of a ferment, called diastase, on starch produces first dextrin and then maltose. This ferment is found in barley and other grains. Maltose is also formed by the action of ferments in the saliva and pancreatic secretion. Dilute sulfuric acid heated with starch paste yields maltose as an intermediate product. By prolonged boiling with water, or by less boiling with dilute mineral acids, it is converted into dextrose. It forms a characteristic osazone with phenyl hydrazin soluble in 75 parts of boiling water. It melts at 206°C. Maltose, when injected into the blood, is eliminated almost entirely unchanged; it is therefore not to any degree assimilable. It is formed, with a small amount of dextrose, by pancreatic digestion of starch, but has been found by experiment to be readily converted into dextrose when allowed to stand with an infusion either of intestinal lining or tissue; hence it is supposed that it is converted into dextrose during absorption.

Iso maltose.

This is an isomer of maltose formed by fermentation of starch. It is more difficulty fermentable than maltose, but by action of diastase yields maltose. It yields an osozone with different melting point (153°) from maltosazone.

Laboratory Work.—Maltose.

Make a starch paste by stirring 1 g. starch into 100 c. c. boiling water. Cool to 60°, add 1 g. powdered malt and keep at 60° for an hour. At intervals of 10 minutes test ½ c. c. of solution with iodine solution and note the results for comparison with action of saliva on starch. At the end of an hour boil and filter. Save the filtrate for tests to compare with sucrose, lactose, and dextose.

Lactose—($C_{12}H_{22}O_{11}+H_2O$).

Lactose is contained in considerable quantities in milk. It is occasionally found in the urine of women in the early days of lactation or after weaning. It is crystallizable, dextro-rotary and less soluble in water than other sugars. It is less sweet than dextrose and its reducing power is 7:10 when compared with that of dextrose. Upon heating with acids it forms dextrose and galactose. If the heating be long continued it will yield lævulinic and formic acids and humin. Upon oxidation with nitric acid, it is first inverted and then the dextrose forms saccharic acid, while galactose forms mucic acid. Bacteria readily cause a fermentation which yields lactic acid ($C_3H_6O_3$). It does not ferment with yeast and does not reduce

Barfoed's reagent. In the latter respect it is like maltose The lactosazone crystals melt at 200°.

Laboratory Work.—Sugars. Try with sucrose, lactose and the prepared maltose solutions tests 5 to 11, inclusive, as given under glucose. Note the results together with those for same tests with glucose, in the following table:

	Glucose.	Sucrose.	Maltose.	Lactose.
5—Trommer's	.....	.....	.....	....
6—Fehling's	.....	.....	.....	....
7—Haines'	.....	.....	.....	....
8—Barfoed's	.....	.....	.....	....
9—Boettger's	.....	.....	.....	....
10—Nylander's	.....	.....	.....	.....
11—Molisch's	.....	.....	.....	.....

Try the fermentation test with glucose, sucrose and maltose.

Boil 50 c. c. of cane sugar solution with 6 to 8 drops concentrated HCl for 2 or 3 minutes. Render alkaline with NaOH and test by 5, 7, 8, 9 and 10. Compare these tests with those made before boiling sucrose with an acid and explain.

POLYSACCHARIDS.

Starch—$(C_6H_{10}O_5)_n$ — Starch is one of the important foods derived from the vegetable kingdom. In many plants it is often stored in roots, bulbs and tubers; in others it forms a large part of the seed, and in still others the stem of the plant contains a deposit of starch granules. A thin slice of a potato will reveal in the cells of the tissue finely striated starch grains, which furnish to this tuber its chief value as a food. Starch grains vary in size and shape, but consist of a material which is sometimes called cellulose, and another portion called granulose. The cellulose differs from that in the walls of the ordinary plant cell, however, as it dissolves in reagents in which ordinary cellulose does not. The granulose and cellulose are arranged in alternate layers. The granulose has the greatest food value.

Starch is insoluble in cold water. It forms a pasty, opalescent mass in boiling water, which is not really a solution, though it may, when diluted, have the appearance of one. The boiling water causes the granules to swell up and burst the envelopes of cellulose, allowing the granulose to escape, which forms the principal part of starch paste. The most characteristic reaction of starch is the blue color formed with iodin solution after having been placed in boiling water. When dry starch is heated to 150°–170° it changes into dextrin. This change is accompanied by a change from white to yellow. This product will form with water a mucilaginous material much used as an adhesive.

Under the influence of diastase, pancreatic juice, or saliva, starch paste is converted successively into amylodextrin (soluble starch), erythrodextrin, achroodextrin, maltodextrin, isomaltose and maltose.

When starch is boiled with dilute sulfuric or hydrochloric acid it passes rapidly through the above changes, but yields, finally, dextrose.

In the body of animals the starch is known to produce fatty tissue. Certain bacteria acting on starch may give rise to fatty acids.

Malt diastase by action on starch produces no dextrose; the pancreatic juice yields but little, and it is still a disputed question whether the saliva produces any dextrose. The chemical formula for starch has not been definitely determined, but it is known to be some multiple of the group $C_6H_{10}O_5$. By an application of Raoult's method the formula for soluble starch is determined as $5(C_{12}H_{20}O_{10})_{20}$.

Laboratory Work.—Starch.—1. Place a very little starch in cold water and take a drop containing some of the starch granules for microscopic examination. Note the shape and markings of starch granules from several different sources. Allow a drop of iodin solution to flow gently under the cover glass; note the color which is thus produced.

2. Moisten a very small amount of starch with two or three drops of water, then pour 10 c.c. boiling water upon it, mix thoroughly and continue heating a moment. To some of the resulting starch paste add a few drops of an iodin solution. The deep blue color is caused by a peculiar effect of iodin on starch. If it is a compound, its composition is not yet known. It is a convenient test for starch.

3. Try to dissolve a small amount of starch in about 10 c.c. of cold water. Filter, boil filtrate and test with iodin solution; will starch dissolve in cold water?

4. Boil one or two c.c. of a soluble starch solution with Fehling's solution. Note that it does not reduce the solution.

5. Boil about one gram of starch in 100 c.c. of water in a small beaker, add 3 or 4 c.c. of dilute H_2SO_4, cover with a watch glass and boil gently. Test a small portion with iodin and with Fehling's solution at intervals. Notice that when erythrodextrin is formed it gives a red color with iodin; that achroodextrin, maltodextrin, isomaltose, maltose and dextrose give no color with iodin; observe also that as erythro-dextrin forms, Fehling's solution is reduced, and the reducing power increases with the boiling until dextrose results. Barfoed's reagent may be used to distinguish the point at which maltose is formed and changed into dextrose, as the latter reduces this reagent, while the former does not.

6. Place one gram of starch in a flask having a capacity of 200 or 300 c.c., add 5 c.c. of strong nitric acid and heat gently under the ventilating hood. When a reaction begins, remove the heat until it has ceased; then evaporate to a small volume and crystals of oxalic acid will appear in the residue upon cooling.

7. To some starch paste add tannic acid; a precipitate is formed, or opacity produced.

The Dextrins—$(C_6H_{10}O_5)_n$ — As stated above, several compounds bear-

ing the name of dextrin are formed by the hydrolysis of starch. They are distinguished by differences in solubility, in rotary power, and in reactions with iodin and other reagents.

Dextrin may be prepared by heating starch to 150° or 160° for a considerable time, or by heating with dilute acids. In digestion it is formed by the action of saliva and by pancreatic juice. It is probably all transformed into dextrose before entering the blood, though in experiments outside the body some dextrin will be unchanged however long the process be continued. Dextrin is not fermented by yeast, but after it is hydrated to maltose the fermentation will occur.

Laboratory Work.— 1. Heat about 5g. of starch to about 155°—just below the point at which it scorches. Stir well and continue heating for ten minutes after the starch is uniformly yellowish brown. Cool, add water, boil and filter.

2. To the filtrate obtained above add tannic acid, no precipitate is is formed, compare with action of soluble starch.

3. To some of the solution add iodin, explain the results by comparing with experiment 5 under starch.

4. To about 4 c.c. Fehling's solution add one c.c. of the solution and boil. Ordinary dextrin reduces Fehling's solution.

Glycogen—$(C_6H_{10}O_5)_n$.—Glycogen is in many respects similar to starch, especially in its hydrolytic changes. It is sometimes called animal starch. Its exact chemical formula is not known,"n" being supposed by some to be 6, by others 10. It was first found in the liver, but was afterwards extracted from muscle and other tissue. It is also found in oysters and some plants.

Glycogen is formed in the liver and distributed from that organ to the blood either as glycogen or as glucose. It is still a debated question in which form it passes into the circulation. The amount in the liver at any time depends upon the kind and quantity of food. Ordinarily the liver contains from 1 to 4 per cent but may contain 12 to 16 per cent after a hearty meal of carbohydrates. Work or starvation causes the glycogen to disappear from the muscle of which it is about 0.6 per cent. After death it rapidly changes into dextrose by fermentation, which fact makes the estimating of its exact amount a difficult process. Some suppose the change from glycogen to dextrose due to a ferment; others, to specific metabolic power of the liver cells.

Glycosuria—diabetes—is due to a disturbed metabolism in the liver causing excretion of glucose in the urine. That which glycogen yields being ordinarily consumed in the body, is in this disease excreted unused. The causes of such a disturbance may be varied—an injury at or near the base of the brain, an injury to the pancreas, nervous exhaustion, or an injury to the spine.

There are ferments found in malt, in the saliva, pancreas, and perhaps in the liver or blood which cause glycogen to pass through the same stages

which starch passes through in hydrolysis, and dextrose results. Some have supposed that the dextrose found in the blood after death results from a post mortem fermentation of glycogen. The diastasic action of ferments is most marked in neutral or slightly acid condition. Glycogen is not affected by yeast. An acid solution of CO_2 hinders the fermentation of glycogen.

Glycogen is a white, amorphous powder which dissolves freely in water and forms usually an opalescent liquid. It is readily precipitated by alcohol when pure, but not from very dilute solutions. A trace of sodium chlorid will cause its precipitation. It gives a red color with iodin as erythrodextrin does, which disappears on warming and reappears on cooling. The dextrins are, however, less readily precipitated by alcohol, requiring at least 85 per cent alcohol, whereas 60 per cent alcohol will precipitate glycogen. It yields precipitates also with tannic acid, barium hydrate and basic lead acetate.

Cellulose $(C_6H_{10}O_5)n$.

Cellulose is the principal constituent of cell walls in plants. It undergoes changes similar to those of starch when heated with mineral acids, but less readily. Materials composed of nearly pure cellulose are cotton fiber and Swedish filter paper. The digestive fluids have little action on cellulose, hence the necessity of boiling starchy foods. The boiling breaks up the envelopes of cellulose allowing the escape of the digestible contents. Cellulose is found in few animals as in the outer covering of the Tunicates.

Under the action of nitric acid, nitro-celluloses are formed. Among these are collodion and gun cotton.

FATS.

Fat as it exists in animal bodies is a combination of an acid group with a glycerol radical. Of these acids, palmitic ($C_{15}H_{31}COOH$), stearic ($C_{17}H_{35}COOH$), and oleic ($C_{17}H_{33}COOH$), are the most common. Olein $C_{17}H_{33}COO)_3C_3H_5$), stearin $(C_{17}H_{35}COO)_3C_3H_5$), and palmitin $(C_{15}H_{31}COO)_3C_3H_5$), are found in human fat. It will be remembered that by the addition of water under high heat, the fats may be decomposed into glycerol and fatty acids.

$$(C_{15}H_{31}COO)_3C_3H_5 + 3H_2O = 3C_{15}H_{31}COOH + C_3H_5(OH)_3.$$

The reaction of superheated steam on the other fats is analagous to this one. It is not an easy matter to unite fatty acids and glycerol to form fat, but it has been done in laboratory experiments. A decomposition similar to that by superheated steam may be made by mineral acids, and in the body by steapsin, the fat-splitting ferment of the pancreatic juice. Saponification of fat also occurs in the small intestines. This process is illustrated in making soap. The reaction with a strong base may be as follows:

$(C_{17}H_{35}COO)_3\ C_3H_5+3KOH=3C_{17}H_{35}\ COOK+C_3H_5\ (OH)_3$.

Emulsification is a division into minute particles distributed through the medium in which they are suspended. The pancreatic juice possesses the property of emulsifying fats. The origin of fat in the human body is a question of great interest. In is now believed that fat results not only from fatty food but from starch, sugars and even proteids. Experiments with animals lead to this conclusion.

Adipocere is a waxy substance consisting of fatty acids and their salts, formed in cadavers buried long in damp earth. The formation of adipocere from proteid matter shows the possibility of fatty acids from such proteids. Lecithin ($C_{44}H_{90}NPO_9$), is a complex fat found in red blood corpuscles, bile, serous fluids, semen, pus, white blood corpuscles, and is more abundant in brain and nerves. It is prepared from the yolk of egg for laboratory experiments but its preparation is complicated. Upon decomposition it yields glycerol, stearic acid, and an alkaloid called "cholin"—$C_5H_{15}NO_2$.

Laboratory Work.—Fat.

1.—Test the solubility of bits of tallow in ether, benzine and alcohol. Use small amounts of the liquid and ascertain which has the least solvent power on fat. Test the reaction of the alcoholic solution by litmus, or alcoholic solution of rosolic acid.

2.—To a small amount of fat crushed into pulp and 5 c. c. of a mixture of equal parts of alcohol and etner; warm *gently*; avoid heating sufficiently to set the vapor on fire; Put a drop of the clear liquid on a glass slide, cover with a cover glass and allow to stand. Examine after a short time with a microscope for fat crystals.

3.—Cut up about 10 g. of pork fat or beef suet into as small pieces as possible. Heat in an evaporating dish at 120° to 130°, stirring constantly. Maintain this temperature for about ten minutes. Strain through a piece of muslin, pressing out the fluid fat. The residue is connective tissue and some fat the latter may be extracted by ether and alcohol.

4.—Place a small quantity of the fluid fat on a piece of filter paper and note its transparency. Try to explain the cause of the transparency.

5.—Rub up thoroughly in a mortar a piece of fat with $KHSO_4$. Transfer to a dry test tube and heat cautiously until a peculiar irritating odor is noticeable. This is due to acrolein, When the formula of glycerol and acrolein are written together for comparison it is easy to understand the source of the acrolein.

Glycerol, $CH_2OH.\ CHOH.\ CH_2OH$.
Acrolein, $CH_2.\ CH.\ COH$.

6.—Mix 5 c. c. of a saturated solution of carbonate of sodium with an equal volume of water; add a small amount of fat obtained in experiment 3; warm and shake thoroughly. The liquid becomes milky but upon standing the fat accumulates at the top. It is not emulsified, saponified or dissolved.

7.—Melt the remaining fat and transfer it to a small Erlenmeyer flask. Add 35 c. c. alcohol and 3 g. of KOH. Insert a stopper with a condensing tube 18 to 24 inches long and heat over a water bath for half an hour. Pour a little of the solution into water; if it remains clear, the saponification is complete. If not complete, continue heating until it is. Write the reaction for the saponification of stearin in this experiment. Preserve the soap solution for following experiments.

8.—Pour about half of the soap solution made in experiment 7 into warm, 3 per cent sulfuric acid. The fatty acids are set free and rise to the surface. Write the reaction. Heat on water bath until the liquid below the fatty acids is clear. Filter through a filter previously moistoned with hot water. What is in the filtrate? What three fatty acids are retained by the filter? Wash the residue with hot water. Preserve the filtrate without the washings.

9.—Dissolve a small portion of the fatty acids in a little alcohol and test with rosolic acid and litmus. What is the reaction? Why do fats become rancid on standing?

10.—With a porton of the fatty acids try the test given under experiment 5. Explain your results.

11.—Repeat experiment 6 using fatty acids instead of fat. What is formed? Warm and add a few drops of olive oil and shake. Is the emulsion permanent? What is now present that was not in experiment 6? Examine a drop of the emulsion under the microscope. What constituents of the pancreatic juice would produce a similar condition?

12.—To one portion of the warm soap solution add calcium chlorid solution. Explain the results.

13. The filtrate of experiment 8 may be concentrated and purified by alcohol and ether and again evaporated to show the presence of glycerol. But a quicker way is to mix 50 c.c. cottonseed oil with 25g. litharge in 100 c.c. water in a porcelain dish and heat with constant stirring until the oil globules have disappeared. When saponification is complete add more hot water, stir well, then allow to settle and filter. Concentrate the mixed filtrates nearly to dryness. Taste the syrupy residue.

14. Mix a little borax with the residue and test it by platinum wire in the flame. Explain the green tinge to the flame.

PROTEIDS.

The proteids occur in all living organisms and seem to be necessary to to the existence of even the simplest forms of life. It is impossible to know whether the same substances exist in the living animal as those obtained from its dead tissues; but some form of proteids may be obtained from every body that has possessed life. Their exact formula has never been de-

termined as their percentage composition seems to vary somewhat. Lieberkuhn suggests $C_{72}H_{112}SN_{16}O_{22}$ as approximate. The percentages according to two distinguished chemists are as follows:

	Hoppe-Seyler.	Drechsel.
C.	51.5——54.5	50.0——55.0
H.	6.9—— 7.3	6.8—— 7.3
N.	15.2——17.0	15.4——18.2
S.	0.3—— 2.0	0.4—— 5.0
O.	20.9——23.5	22.8——24.1

The different members differ in solubility, consistency and various physical and chemical characters. There are, however, many reactions in common as well as a very similar percentage composition.

Upon decomposition so many different substances are obtained that the molecule must be very complex, but no definite structure can be ascertained. The decompositions in the body are so different from those in the laboratory that it has led to the belief that the constitution of living protoplasm is different from that obtained after death. According to Halliburton's Kirke the chief final decomposition products of proteids are carbon di-oxid, water and urea. Glycocin, leucin, creatin and uric acid are probably intermediate products. Carbohydrates and fats may be formed in the body from proteids. Outside of the body they may be broken up into ammonia, carbon di-oxid, amins, fatty acids, amido acids (like leucin), lysatin, glycosin and aromatic compounds like tyrosin. The student will determine their solubilities in alcohol, ether, water, various salt solutions, and acids.

Most proteids coagulate when heated, though at different degrees. Proteids (peptones excepted) will not pass readily through animal membranes. The processes of digestion transform them so that they are capable of absorption. Bodies which will not pass through animal membranes are called colloids.

Classificasion of Proteids.

The following classification is that of Hoppe-Seyler:

I. *Native Albumins.* Egg-albumin, Serum-albumin.

II. *Derived Albumins.* 1. Acid-albumin; 2. Syntonin; 3. Alkali-albumin; 4. Casein or Native Alkali-albumin.

III. *Globulins.* 1. Crystallin, the globulin of the crystalline lens; 2. Vitellin. 3. Paraglobulin, or Serum-globulin; 4. Fibrinogen; 5. Myosin; 6. Globin.

IV. *Fibrins.*

V. *Coagulated Proteids.*

VI. *Albumoses and Peptones.*

VII. *Lardacein, or Amyloid Substances.*

Class I. Native Albumins.

Egg Albumin and Serum Albumin are examples. They are soluble in

distilled water, coagulate upon heating, especially in the presence of dilute acetic acid. They are not precipitated by carbonates of alkalis, by sodium chlorid, or generally by neutral salts.

Serum Albumin differs from Egg Albumin in several respects.

1. It is not so readily coagulated by alcohol or precipitated by ether as egg albumin is.

2. It is not readily precipitated by strong hydrochloric acid and the precipitate is readily soluble in excess of HCl; the reverse is true of egg albumin.

3. Precipitated or coagulated serum albumin is more readily dissolved in nitric acid than is egg albumin.

4. Egg albumin, if injected subcutaneously, or into a vein, reappears unaltered in the urine; serum albumin will not normally be excreted in the urine, if injected into the blood.

Laboratory Work,—Egg Albumin and General Proteid Reactions.

Cut or beat up the white of an egg until the fibers are broken; divide into four parts. To the first part add four parts water; to the second part add nine parts water; to the third part add forty-nine parts water; preserve the fourth part undiluted. The diluted portions should each be mixed well with the water added and then filtered.

1.—Place 1 c. c. of the undiluted white of egg in a test tube; heat water in a water bath and stir it with the test tube containing the egg albumin and a thermometer whose bulb is immersed in the albumin. Note the temperature at which it coagulates.

2.—Mark three test tubes with numbers 1, 2, and 3 respectively. In number 1 place 5 c. c. 1-5 albumin solution; in number 2 place 1-10 albumin solution; in number 3 place 1-50 albumin solution. Immerse the three three tubes in boiling water in a water bath for five or ten minutes; observe the results. They become opalescent, but do not coagulate. How does dilution affect coagulation of egg albumin? Preserve the tubes and contents for comparison with the next experiment.

3.—Mark 3 more tubes with numbers 2, 3, 3a, respectively. In number 2 put 5 c. c. 1-10 albumin solution and 1 c. c. of 10 per cent salt solution; in number 3 put 5 c. c. of 1-50 albumin solution and 1 c. c. of 10 per cent salt solution; into 3a put 5 c. c. of 1-50 albumin solution and 0. 2 c. c. of 10 per cent salt solution. Immerse in boiling water for five minutes. Compare tubes 2 and 3 with tubes 2 and 3 of previous experiment. The coagulation will probably be noticeable, while in tube 3a the opalesence will be greater than in tube 3 of the previous experiment. Now to the three tubes containing the salt and albumin add one or two drops of 1 per cent acetic acid and heat again. A complete precipitation in all three tubes results. What conclusion may be drawn from a comparison of these experiments with the preceding?

4.—In each of two tubes place 5 c. c. of 1-50 albumin solution. To one

add one drop of 1 per cent acetic acid; to 2 add five drops 1 per cent acetic acid. Immerse in water bath 5 minutes. In number 1 the albumin is completely precipitated; in number 2 it has been changed to acid albuminate (Class II.), and dissolved. Now, to each day add 1 c. c. of 10 per cent NaCl solution and heat again. Complete precipitation results. What conclusion may be drawn?

5.—To 1 c. c. of 1-5 egg albumin solution add several times the amount of alcohol and shake vigorously. The alcohol causes coagulation. Try a more dilute solution (1-50), and after adding alcohol, observe; then add 5 c. c. 10 per cent salt solution.

6.—To about 3 c. c. of egg albumin solution (1-50), add an equal volume of concentrated nitric acid. Let the acid glide gently down the side of the test tube so that the acid will settle to the bottom without mixing. At the surface of the acid a cloudy ring of precipitated albumin will be formed, (Heller's test). Now mix the liquids and warm gently; a precipitate remains. Boil the mixture for a short time and the albumin will dissolve forming a yellow liquid containing acid albumin. Add 2 c. c. of ammonia and an orange color will appear. This is called the "*Xanthoproteic* reaction." Serum albumin will not dissolve so readily in excess when precipitated by nitric acid, hence a combination of the Heller test and subsequent heating, is a valuable test for albumin in urine.

7.—Saturate 10 c. c. of dilute albumin solution with $(NH_4)_2 SO_4$. This may be done by heating at 35° in a water bath for half an hour with sufficient $(NH_4)_2 SO_4$ added. Test filtrate by acetic acid and heat. Is albumin completely precipitated?

8.—Saturate 10 c. c. of dilute albumin solution with $MgSO_4$, using 12g. and the same method as in experiment 7. Only a slight cloud forms, due to the globulin. Test the filtrate as above. How may albumin be separated from globulin?

Alkaloidal Tests.

9.—To about 5 c. c. albumin solution add one or two drops strong acetic acid then add two or three drops of potassium ferrocyanid. A precipitate forms. If much potassium ferrocyanid is used the liquid imparts a yellowish color to the precipitate which is otherwise white. This test is not given by peptones.

10.—To 5 c. c. albumin solution 1-50, add a few drops of strong HCl and then a few drops of phosphotungstic acid (H_3PO_4 $11WO_3$). This is given by all proteids. Phosphomolybdic acid (H_3 PO_4 $11MO_3$) acts in the same manner.

11.—To 5 c. c. albumin solution, 1-50, add a few drops of strong HCl and then add a few drops of potassium mercuric iodid. (Formula next to last page.)

12—Add a few drops of tannic acid to 5 c. c. dilute albumin solution. Recall action of tannic acid on starch and dextrin.

13.—Add a few drops of picric acid to a portion of dilute albumin solution.

Tests With Heavy Metals.

14.—To a portion of dilute albumin solution add 8 or 10 drops of a solution of silver nitrate; to another an equal amount of lead acetate; and to another an equal amount of mercuric chlorid. Why is white of egg an antidote for corrosive sublimate poisoning? Why should a stomach pump be used after the administration of white of egg?

Color Reactions.

15.—Millon's Reaction.—To some 1-50 albumin solution add a few drops of Millon's reagent, and boil. The white precipitate formed at first turns red by boiling.

(15)—Liquid also becomes red. Reaction caused by phenol group. Formula for millions reagent on the last page.

16.—Biuret Test.—To 8 c. c. 1-50 albumin solution add equal volume of NaOH solution. Then heat to boiling and add one to two drops of copper sulfate solution. A pink or violet color appears. Avoid excess of $CuSO_4$. This reaction may indicate that proteids contain the biuret group.

Or it may indicate a diamid group as this group will also give the biuret reaction. If a diamid group is removed from the proteids it will not give the biuret reaction. The relation of the biuret group to urea may be shown by the following reaction.

$$(2 \text{ mols. urea}) \begin{matrix} CO \begin{matrix} NH_2 \\ NH_2 \end{matrix} \\ CO \begin{matrix} NH_2 \\ NH_2 \end{matrix} \end{matrix} \text{ minus } NH_3 = (\text{Biuret group}) \begin{matrix} CO \begin{matrix} NH_2 \\ NH \end{matrix} \\ CO \begin{matrix} \\ NH_2 \end{matrix} \end{matrix}$$

17.—Adamkiewiczs' Reaction.—To 1 c. c. of H_2SO_4 add 2 c. c. of glacial acetic acid and mix. To the mixture add 1 drop dilute egg albumin. Warm gently, allow to stand; a reddish violet color appears. Reaction shows presence of aromatic group.

As the tests are tried with various kinds of proteids indicate by a -|- or — sign in the following table, whether a precipitate is obtained. If any peculiarities, indicate on the blank page opposite.

Laboratory Work—Serum Albumin.

A quantity of blood collected at the slaughter house should be whipped with a bundle of twigs or an egg beater, until the fibrin is removed. The corpuscles should be allowed to settle, or be separated in the centrifuge, and the clear serum used to separate albumin and globulin from. The first two experiments may be tried simultaneously.

1. Take 10 c.c. of serum, add an equal volume of solution of $MgSO_4$; also 15g. powdered $MgSO_4$; continue stirrring and heat 30 minutes at a temperature between 30° and 35°. This will saturate the entire fluid and precipitate the globulin. Filter and save filtrate to test for serum albumin by tests in above table. If $MgSO_4$ is present, it will interfere with test No. 14 in the table. Why? When sodium hydroxid is added to make biuret test,

will excess of alkali dissolve the MgOH formed? After washing the globulin on the filter with $MgSO_4$ solution, remove the precipitate and dissolve in 20 c. c. water and perserve with proper label. Globulin will not dissolve in water, ordinarily, but does in this case because of neutral salts present.

2. Globulin is precipitated from a mixture with albumin by semi-saturating with $(NH_4)_2SO_4$. To 10 c. c. of blood serum add 10 c. c. saturated ammonium sulfate solution. Immerse in water bath at 30° to 35°, stirring frequently, for 30 minutes or longer. Filter and save filtrate to test serum albumin according to table at close of section on native albumins. The residue should be washed with semi-saturated solution of ammonium sulfate. After washing, dissolve the residue in 20 c. c. of water as the amount of ammoniun sulfate is sufficient to cause its solution in that quantity of water. This filtrate containing globulin can be placed in a dialyzer with that obtained in the last experiment and preserved for experiments on globulin.

3. To 10 c. c. of blood serum add an equal volume of saturated solution of $(NH_4)_2SO_4$. Then add 8g. of $(NH_4)_2SO_4$ and warm at 35° or 40° for half an hour. This saturates the solution and causes a precipitate. Filter and test filtrate by boiling, by tannic acid, and by biuret test in the cold. The absence of proteids indicates complete precipitation of albumin and globulin; and the negative results from biuret test show absence of peptone.

Class II. Derived Albumins.

Derived albumins are nearly insoluble in pure water or dilute saline solutions. They are soluble in acids and alkalies. They do not coagulate by boiling.

Laboratory Work—Derived Albumin.

To 5 c. c. of egg albumin in a small beaker add a strong solution of sodium hydroxid until a thick jelly forms. Avoid an excess of alkali. Cut in pieces the jelly-like material and wash with cold, distilled water. Heat some of the pieces gently in distilled water until dissolved. Filter and precipitate from filtrate with very little acetic acid and wash with pure water. Test solubility in dilute acids and alkalis.

2. Take some of the washed alkali albumin obtained in experiment 1 and dissolve in hot water; add an indicator, as phenol phthalein solution, a few drops, and exactly neutralize with sulphuric acid. When the neutral point is reached a precipitate is formed, which dissolves in either excess of acid or alkali.

The jelly formed in experiment 1 contains excess of alkali and is called "Lieberkuehn's jelly." Why does this alkali albumin dissolve in water in view of the statement made previously that it is insoluble in water.

Acid Albumin.

Acid albumin is less soluble than alkali albumin and seems to have a

different rotary effect on polarized light. It may be prepared by warming the white of egg with dilute acid solution.

1. To 25 c. c. of a mixture of one part white of egg and four parts water add 5 c. c. of 0.2 per cent hydrochloric acid and warm at 45° for about two hours. Carefully neutralize with dilute sodium hydroxid. If exactly neutral, acid albumin is precipitated, which may be washed by decantation.

2. Redissolve a portion in dilute HCl. Observe that it is not coagulated by boiling.

3. Mix a portion in water and heat to 75° or 80°. The precipitate is changed to coagulum.

4. Try Millon's test, the biuret test, and the xanthoproteic reaction, with portions of the acid albumin.

Casein.

Pure casein is a white powder almost insoluble in water, but soluble in alkalis and acids. From such solutions it may be precipitated by excess of neutral salts and in dilute acids. If acids be added in excess, it dissolves. Solutions of pure casein are not coagulated by boiling. Casein contains a nearly constant proportion of phosphorus and is therefore classified by Hammarsten as a nucleo-proteid. Casein clots with rennin forming a compound different from the casein. Clotting will not occur in the absence of all calcium salts, unless magnesium, barium, or strontium salts are substituted. It has been supposed that casein was identical with alkali albumin, but the evidence is against such a supposition. Pure rennin will not coagulate alkali-albumin. Pure casein yields no ash on ignition. It has been supposed that casein is derived from an antecedent called caseinogen.

I. To 50 c. c. of water add 10 c. c. of milk and mix. Add enough acetic acid to give a distinctly acid reaction, avoid excess. Allow to settle and wash by decantation. Filter, drain and squeeze dry, and wash with 10 c. c. of alcohol. Pour off the alcohol, add 25 c. c. of ether, place in small flask and after inserting condensing tube heat for ten minutes to boiling, on a water bath. The fat is dissolved by this treatment and may be obtained from the alcohol and ether filtrates by evaporating on a water bath which has been heated to boiling and the flame extinguished.

II. Test solubilities of casein in alkalis, in acids and in distilled water. In what respects is it similar in these tests to alkali and acid-albumin?

III. Mix 10 c.c. of milk with 5g. of powdered $MgSO_4$ and shake vigorously. Let stand one hour. Casein is precipitated. Boil the filtrate and notice the coagulation of albumin. Milk contains albumin and globulin which are similar to those in blood, if not identical. They are sometimes called lactoglobulin and lactalbumin.

Syntonin.—This substance is an acid albumin derived from the action of dilute acid on myosin. It is, however, so different from the acid-albumins obtained from other sources that it deserves separate consideration. It is insoluble in di-sodic phosphate; other acid-albumins are soluble. It may be reconverted into globulin similar to myosin.

Laboratory Work—Syntonin. 1. Take some of the muscular part of meat freed from fat and ground fine in a sausage mill, and wash with water until the washings remain clear. To 5g. of the moist residue in a small flask, add 50 c.c. of 0.1 per cent HCl. Warm to between 35° and 40° and keep at that temperature about three hours. Filter, and in the filtrate will be syntonin.

2. Neutralize a portion by alkali (NaOH), syntonin is precipitated. An excess of alkali dissolves as in other albumins.

Class III. Globulins.

The globulins, like the derived albumins, are not soluble in distilled water. They are, however, soluble in water containing small quantities of neutral salts, but most of them are insoluble in excess of these salts. They are soluble in dilute acids and alkalis, forming acid aud alkali albumins, respectively. Like the native albumins, they coagulate with heat and are precipitated by alcohol.

Crystallin. This one of the globulins occurs in the crystalline lens of which it constitutes nearly 25 per cent. It may be prepared by treating the finely divided portions of crystalline lenses with a salt solution, which dissolves it. It may be precipitated from the salt solution by diluting with distilled water and saturating with carbonic anhydrid.

Vitellin. The characteristic proteid constituent of egg yolk is vitellin. It may be obtained mixed with lecithin by the extraction of the yolk of eggs with ether. When so obtained it is a white, pasty, granular mass, insoluble in water and readily soluble in solution of sodium chlorid. Excess of sodium chlorid fails to precipitate vitellin as it does other globulins. It is soluble in sodium carbonate (1 per cent) solutions and is precipitated by diluting and passing CO_2 gas through the mixture. It is associated with nuclein and is regarded by some as a nucleo-proteid.

Paraglobulin, (Serum-globulin).

Blood serum or lymph contains paraglobulin. It is now supposed to constitute one half of the serum proteids. It is insoluble in pure water, but is dissolved by the addition of even a small amount of neutral salts. It may be separated from the albumin of blood serum by saturation with $MgSO_4$, or by semi-saturation with $(NH_4)_2SO_4$.

Laboratory Work—Paraglobulin.

Take the globulin obtained by the dialysis of blood serum in experiment 2 under "Serum Albumin," and dissolve in a 2 per cent salt solution. This may be done by agitating the mixture of globulin and salt solution and allowing to stand for a time. Observe that the liquid froths when shaken.

1. Heat a small portion of the globulin solution. Does it coagulate? Filter, and to the filtrate apply the biuret test. Was the coagulation complete?

2. Try the tests and fill out the blank spaces under serum globulin in

the table at close for general tests for proteids. Observe that some tests will be interfered with by the presence of NaCl. Let the student indicate these tests in his notes. By reference to experiment 3 under "Serum Albumin," the student will recall that both serum globulin and serum albumin are precipitated by complete saturation with $(NH_4)_2SO_4$.

Fibrinogen.

The substance in the blood plasma, which by clotting yields fibrin, is called fibrinogen. It is also found in chyle, serous fluids and transudations, such as hydrocele fluids. It is distinguished from paraglobulin (1) by forming clots spontaneously from plasma, (2) by coagulating in 16 per cent salt solution while paraglobulin does not appreciably coagulate in a solution below 20 per cent. The change from fibrinogen to fibrin is not yet well understood but may be due to a ferment called fibrin ferment present in the blood.

Myosin.

When death occurs, the pliable muscles undergo a change called rigor mortis. An almost fluid constituent resembling fibrinogen forms a less fluid substance called myosin. The name myosinogen is now generally accepted for the antecedent of myosin. Myosin coagulates at 55° to 56° in salt solutions. Upon ignition it leaves a large ash residue largely composed of salts of lime. It is converted into syntonin by the action of acids.

Laboratory Work—Myosin.

1. First secure some muscle from round steak; free it from fat, grind it in a sausage mill until it is finely pulverized, and wash it until the wash water is clear. Place it in a 20 per cent solution of ammonium chlorid and stir well. Allow to stand about 24 hours with occasional stirring or shaking. The myosin dissolves and upon filtering will pass into the filtrate. Pour the filtrate into 20 volumes of distilled water, which will cause a precipitation of myosin. Allow to settle and wash three times by decantation.

2. Dissolve a portion in 10 per cent solution of common salt. Ascertain if it can be precipitated by saturation with common salt.

3. Test its solubility in 0.1 per cent solution of HCl.

No. Test.	Egg Albumin	Serum Albumin	Serum Globulin	Peptone	Gelatin
5.—Alcohol	.....	.....	.....	.....	.....
6.—Nitric Acid	.....	.....	.....	.....	.....
7.—$(NH)_2SO_4$	.....	.....	.....	.....	.....
8.—$MgSO_4$ (Sat.) ...	.....	.....	.....	.....	.....
9.–Ac.acid&Pot.F.Cy.	.....	.....	.....	.....	.....
10–Phospho tungstic.	.....	.....	.....	.....	.....
11–Pot. Mercu. Iodid.	.....	.....	.....	.....	.....
12–Tannic acid	.....	.....	.....	.....	.....
13–Picric acid	.....	.....	.....	.....	.....
14–Heavy Metals	.....	.....	.....	.....	.....
15–Millon's Test	.....	.....	.....	.....	.....
16–Biuret Test	.....	.....	.....	.....	.....

Globin.

When the hemaglobin of the blood undergoes spontaneous decomposition a substance called globin is formed. Globin is similar to the globulins, but not so readily soluble in salt solutions, in dilute acids or in alkalis. It has not been investigated as fully as other proteids.

Class IV. Fibrin.

This proteid forms in threads through blood as it spontaneously clots and entangles the blood corpuscles in its meshes. It may be removed as rapidly as it forms by beating the fresh blood with bundles of sticks. When so removed and the blood corpuscles are washed out, it is a white, elastic substance. It dissolves gradually in acids and alkalies, but is insoluble in water and in dilute saline solutions. Fibrinogen is the primary form in which fibrin occurs in the blood. Experiments on digestion of fibrin will be given in the chapters on stomach and intestinal digestion.

Class V. Coagulated Proteids.

This class of substances is produced by the action of heat on many proteids previously described. The prolonged action of alcohol coagulates all proteids except peptones. The coagulated proteids are not soluble in water in dilute acids or alkalis. When dissolved in strong acids they are broken up. Some writers class fibrin, myosin, and casein in this class; but the difference in solubilities and action with reagents is so great that this classification seems to conform better to these reactions. The coagulated proteids are acted upon by gastric or pancreatic fluids and converted into peptones.

Class VI. Albumoses and Peptones.

In the earlier stages of stomach digestion albumoses are formed, later these are transformed into peptones. Pepsin will not carry the process farther. Pancreatic juice will, however, cause a further breaking up of a part of the peptones and the formation of leucin and tyrosin.

Laboratory Work—Albumoses.

1.—Make a 20 per cent solution of a commercial peptone. It contains both albumoses and peptones. Boil a small amount—3 or 4 c. c.—in a test tube and observe that no coagulation takes place. This shows absence of albumin and globulin.

2.—Saturate 10 c. c. of the solution by adding 10 c. c. of a saturated solution $(NH_4)_2SO_4$ and 8g. of the crystalline $(NH_4)_2SO_4$ and heating one half hour at a temperature 35° to 40°. The sticky precipitate formed consists of albumoses. Filter and save filtrate to test for peptones. Remove the albumoses from the filter and dissolve in about 20 c. c. of water. The solution may be hastened by warming.

3.—Heat a portion obtained above and containing albumoses, to boiling, it does not coagulate.

4.—To another portion add nitric acid drop by drop; a precipitate may form, but dissolves on addition of more acid. When the precipitate is dis-

solved add some saturated NaCl until a precipitate forms. Upon heating, the precipitate dissolves, and when cooled reappears again; characteristic of albumoses.

5.—Try the potassium ferrocyanid and acetic acid test; if no precipitate occurs add saturated salt solution. The precipitate will dissolve on gentle heat, (50° to 60°) and reappear when cold.

6.—Add one or two drops of acetic acid and some saturated salt solution. On heating, the precipitate disappears; and upon cooling reappears.

7.—Try tests in table at close of section on native albumins.

Laboratory Work—Peptones.

1.—To a portion of the filtrate obtained in experiment 1, under albumoses apply the biuret test in the cold. If positive results are obtained, peptone is present.

2.—To the rest of the filtrate obtained above apply the tests in the table under the section on native albumins.

NITROGENOUS BODIES ALLIED TO PROTEIDS.

The substances called by some "albuminoids" have nearly the same percentage composition as proteids and resemble them in many general points. They possess greater difference of properties than do proteids, but may yield leucin and sometimes tyrosin when they undergo hydrolytic decomposition. None of them are crystalline.

Mucin.

Mucin is the substance which gives to many secretions their peculiar viscid consistency,—as in saliva, the synovial fluid and bile. It may be dissolved from the glands, tendons and various connective tissues. There are several varieties of mucin. They are all tenacious. They are also precipitated by acetic acid, but are soluble in dilute alkalis. They are supposed by some to be compounds of a proteid and a carbohydrate called "animal gum;" while others regard their structures as an unsettled question. The animal gum may be simply an accompanying substance, or may be a decomposition product, and not an original constituent.

Collagen.

The fibrils of the white fibrous tissue are composed of a substance called collagen. The same substance furnishes the greater part of the animal matter of the bones; but in the latter place is called ossein. From connective tissue it may be secured by digesting the other constituents of tendons in trypsin. As thus prepared it will not dissolve in water, saline solutions, or dilute cold mineral acids, or alkalis. Upon prolonged boiling with water, or for a shorter time with dilute mineral acids, collagen (ossein) is transformed into gelatin.

Laboratory Work—Collagen.

1.—Take a slender bone and after cleaning thoroughly, place in a 10 per cent solution of HCl. Allow it to stand for about 48 hours. At the end of that time it will be found that the hard parts of the bone have been dissolved. The soft, flexible substance is ossein, identical with the collagen obtained from connective tissue. (Preserve the solution to test for mineral constituents.)

2.—Wash the elastic mass obtained in experiment 1 and boil in pure water until a jelly-like mass is formed. This mass is gelatin.

3.—Take a portion of the acid in which the bone was dissolved and test for calcium. Test another portion for phosphates. What has already shown (in example 1) the presence of the carbonate group?

Gelatin.

When dry, gelatin is a brittle, transparent, somewhat colored substance. It does not dissolve in cold water, but swells up into a spongy mass. Upon heating in water it dissolves and when the liquid cools it forms a jelly-like mass, even when so small an amount as 1 per cent is present. It dissolves readily in dilute acids even when cold. It digests in the gastric juice forming a peptone-like substance, but is not a substitute for proteids as a food. It contains more nitrogen and less carbon than proteids, but is said, when pure, to contain no sulfur. It gives many of the general reactions for proteids.

Laboratory Work—Gelatin.

1—Make a 2 per cent solution of the best French gelatin and to a portion add some bromine water; a yellow, sticky precipitate forms.

2.—Try the tests in the table under native albumins and fill out the blanks.

It will be noticed that the heavy metals do not precipitate gelatin and that the xanthoproteic reaction is weak, the latter indicating the absence of the phenol group. $HgCl_2$ solution will precipitate gelatin from acid solutions. It is probable that the reactions will be affected by the presence of traces of proteids in the solution.

Chondrin.

As collagen is the antecedent of gelatin, so the hyaline matrix of cartilage is the antecedent of chondrin; and the name chondrigen has been proposed for it. Digestion in hot water transforms the chondrigen into chondrin. It dissolves like gelatin in hot water, but unlike gelatin is precipitated by acetic acid, and by a trace of dilute mineral acids, though in the latter it dissolves in excess. It is not precipitated by $HgCl_2$ and is by lead acctate. In these reactions it acts the reverse of gelatin. Chondrin is probably a mixture of gelatin and mucinoid material.

Elastin.

This substance is found is yellow elastic fibers. It dissolves in boiling alkalis, and in boiling mineral acids. Like gelatin, it swells up, but does

not dissolve in cold water. It does not dissolve in hot water, but is soluble in caustic soda. It is also dissolved by strong minerals acids, but in so doing undergoes a chemical change. Elastin is readily digested by pepsin or by trypsin. In undergoing decomposition in strong HCl and $ZnCl_2$ it yields products unlike those given by gelatin or proteids.

Keratin.

A substance obtained from horn, hair and nails, having a texture which imparts to these tissues their characteristic hardness, is called keratin. It is very insoluble. It dissolves in strong, hot alkalis. Its composition is similar to that of proteids, but contains a varying amount of sulfur (.5 per cent to 5 per cent). It is digested by neither pepsin nor trypsin. Upon decomposition it yields leucin and tyrosin.

Neurokeratin.

This is a substance obtained from neuroglia and nerve fibers. It resists decomposition by some of the agents which will decompose keratin, but is in other respects similar.

SALIVA.

The saliva is the product of the three salivary glands—parotid, submaxillary and sublingual, together with the secretions of the serous and mucous glands distributed over the surface of the mouth and tongue. According to the researches of Heidenhain there are two kinds of secreting cells in the salivary glands—albuminous and mucous. The albuminous secretes a more fluid substance containing a small amount of proteid coagulable by heat. The parotid gland is chiefly composed of albuminous cells. The mucous glands secrete a sticky substance containing mucin instead of a proteid. The submaxillary gland is composed partly of mucous cells and partly of albuminous cells the sublingual is composed chiefly of mucous cells. The mixed saliva is, when fresh, a clear, viscid fluid with ordinarily a slightly alkaline reaction. It contains an occasional epithelial cell and a few corpuscles, so similar to leucocytes that they are supposed to be migrating leucocytes somewhat changed. They are more globular and contain minute granules which exhibit the Brownian movement. It may contain bacteria of several varieties and even pus or blood corpuscles in inflammation.

Saliva is ordinarily alkaline though the alkalinity is much diminished, or it may be even acid, after a long fast. In the night, or long after a meal, or after long talking, the saliva may be acid. Saliva contains only five or six parts per 1000 of solid constituents, the rest being water. The specific gravity is from 1.002 to 1.008. The amount of saliva secreted cannot be accurately determined. Mitscherlich estimated it to be 240 to 300 c. c. per

day; others place it at 1400 to 1500 c. c. The quantity is dimished in acute febrile attacks, in diabetes, in severe diarrhoea and in cholera. There is an increase of saliva in certain diseases, especially those which produce an inflamed condition of the mouth, tonsils, and pharynx. Potassium iodid, pilocarpin, mercury salts, acids, alkalis and other irritant poisons increase the flow of saliva. There is a change in the saliva when the flow is increased by drugs,—albumin and salts are increased. The flow of saliva increases after meals and during mastication. A decrease is caused by atropin. There is no dextrose in the saliva in diabetes. In jaundice or other diseases in which bile is apt to be found in the urine, it is not found in the saliva. Urea may be found in the saliva normally in small amount, and in larger amount in cases of uremia. Lactic acid may, in diseased conditions, be found in the saliva.

The saline constituents of the saliva are phosphates, carbonates, sulfates and chlorids combined with sodium, calcium and magnesium. The sodic carbonate and phosphate sometimes form calculi in the ducts of the glands. The tartar on the teeth is of essentially the same composition as the calculi. The calcium salts are held in solution by CO_2; upon standing, this gas passes off and the calcium salts precipitate, rendering the saliva turbid. Potassium sulfocyanate constitutes about .06 per cent of the saliva. It probably results from the decomposition of the proteids in the body.

Nitrites and ammonia are present in the saliva. The most important constituent of the saliva from a physiological point of view is the enzyme, or ferment, called ptyalin. The ferment is diastasic or amylolytic in its action—that is, it acts on starch to cause hydrolytic decomposition, converting the starch into dextrins, then into isomaltose, and finally into maltose. The action seems to be identical with that of diastase which is stored in the grains, especially barley. The temperature at which it acts is, however, different, proving that the ferments are different. The amount of ptyalin present in the saliva varies somewhat. The ptyalin is most active in neutral or nearly neutral solutions. A slight alkalinity is favorable to its action, a trace of acid does not completely arrest its action, but stronger alkalies or decided acidity destroys the activity of the enzyme.

Laboratory Work—Saliva.

1.—Collect about 100 c. c. of saliva. Chewing a piece of clean rubber or pure paraffin will facilitate the flow of the fluid. Test the reaction with litmus.

2.—Test the specific gravity by glacing about 40 or 50 c. c. in a cylindrical graduate and placing a urinometer in it. Any foam should be first removed from the surface with a piece of filter paper. Compare with the reading of water.

3.—To about 5 c. c. of saliva add a few drops of acetic acid; a precipitate of mucus is thereby formed.

4.—Test a portion for the biuret reaction.

5. To a portion add a few drops of nitric acid and heat to boiling. Is albumin present? Add ammonia to ascertain if there is a response to the xanthoproteic test.

6. Observe that mucin in the saliva gives Millon's reaction.

7. To about 5 c. c. of saliva add a drop or two of dilute HCl; shake well and filter; then add drop by drop a dilute solution of ferric chlorid until a red color results;—this is caused by the sulfocyanates in the saliva. In testing the contents of the stomach for opium by a similar test, mercuric chlorid should be added, causing the red color to disappear if due to ferric sulfocyanate.

8. To another portion of the saliva add a little iodic acid and some dilute starch paste. Some iodin will be set free by the sulfocyanate and color the starch blue.

9. To some saliva add a few drops of H_2SO_4 and mix; then add a few drops of potassium iodid solution and some starch paste. Iodin will be liberated by the nitrous acid and the blue color will appear.

10. Another test for nitrous acid is to add a drop or two of HCl and two or three drops of a saturated solution of sulfanilic acid. Upon the further addition of a few drops of naphthylamin hydrochlorid, a pink or red color will appear if nitrous acid is present. This test is much used to ascertain the presence of nitrates in drinking water.

11. The mucin may be precipitated from 5 c. c. of saliva by 25 c. c. of absolute alcohol. After standing a few hours it may be filtered, washed with alcohol and then with ether, and finally dried.

12. It will be found to be insoluble in cold water, but soluble in alkalis and in acid (HCl) by boiling. In the latter case it will reduce Fehling's solution. It is not a sugar, however, though its character is not yet fully determined. (The student will remember that a solution must be rendered alkaline before testing with Fehling's solution.)

13. To about 30 c. c. of starch paste, at a temperature of 35° to 40°, add six drops of saliva. Have a few test tubes with Fehling's solution and others with a small quantity of iodin solution ready to test the mixture at intervals of two minutes. The iodin tests will reveal the changes of starch to dextrins until achroodextrin is formed; while Fehling's test will show the appearance of maltose and its increase. Note time and results of each test.

14. To 10 c. c. of starch solution add 5 c. c. of the saliva and test as in experiment 13. Test rapidly.

15. Boil 5 c. c. of saliva; allow it to cool and add 10 c. c. of starch solution. Test as before with iodin, also with Fehling's solution. What is the result?

16. To 10 c. c. of a starch solution add 0.2 c. c. of a 1 per cent solution of acetic acid; mix and add 3 drops of saliva and mix again; compare the rapidity of the action with that in experiments 13.

17. Repeat experiment 16, using 0.6 c. c. of a 0.3 per cent HCl solution. The HCl solution may be prepared by adding 10 c. c. of concentrated HCl to one liter of water. This will have only about one-tenth of the acidity of the gastric juice.

GASTRIC JUICE.

Its Physical and Chemical Characters.

Pure gastric juice is a thin, usually colorless fluid, possessed of a very acid reaction, and of a peculiar odor. Its specific gravity varies from 1.001 to 1.010, even in the same individual. This variation is dependent upon the conditions of secretion. Boiling the gastric juice does not cause its coagulation, but renders it inactive. The gastric juice of warm-blooded animals ceases to exert its power when cooled to 0°C. The gastric juice of man contains less than 1 per cent of solid matter, of which about ⅔ are organic and ⅓ mineral, There are usually gland cells, epithelial cells and mucous corpuscles present.

The acid reaction of the gastric juice is due chiefly to free HCl, especially when there is no food in the stomach. After the introduction of food, particularly of hydro-carbons, lactic acid appears in considerable quantities, also butyric and acetic acids. Alkaline chlorids, earthy phosphates and iron are found in the gastric juice. The amount of HCl found on the average, in human gastric juice, is from 2 to 3 parts per thousand. It is thought by some to be in part loosely combined with an organic body. The perfectly fresh gastric juice seems to have a very little coagulable albumin, but after standing for a short time only, albumoses can be found. Among the organic materials of human gastric juice are at least two enzymes,—pepsin and rennin. As the gastric juice obtainable is almost always mingled with saliva and residues of food, it is impossible to make perfectly reliable analyses.

Pepsin is the enzyme which acts on the proteid constituents of food and transforms them into peptones and albumoses. It acts only in acid solutions but will act in other acids besides HCl. The gastric juice may be kept for months without losing its proteolytic activity, as it seems to possess strong antiseptic powers. Pepsin does not seem to be a true proteid, as the purest pepsin does not respond to the proteid reactions. It is soluble in water and glycerol. A quite active solution may be made by allowing the minced lining of a hog's stomach to remain two or three days in glycerol. Pepsin acts best at a temperature of 40°, is destroyed at a temperature of 55° in alkaline solutions, at 65° in acid solutions, and at 100° in water. In the dry condition it will endure a temperature of more than 100° without having its power destroyed. A dilute alcohol precipitates it, though it does not render it wholly inert; but an excess of alcohol destroys the activity of the pepsin. Raw fibrin swells up in dilute (0.1 per cent) HCl, but will not

dissolve at ordinary room temperature in two days. Upon the addition of a little pepsin it quickly dissolves. The white of egg cooked will not be changed materially in several hours by a 0.1 per cent solution of HCl, but it will become transparent on the edges, swell up, and gradually dissolve if pepsin is present at the same time. Proteid and similar compounds, except keratin and nuclein are digested by pepsin and HCl. The nuclei of cells and epidermal tissue are therefore the chief nitrogen—containing structures not affected by the gastric juice. Fat is not dissolved, but its envelope of connective tissue is digested. Starch, cellulose and the sugars are not changed by the action of pepsin. The proteids are changed to albumoses and peptones; and even continued action will not form the further decomposition products which result from intestinal digestion.

Rennin is the enzyme which causes the coagulation of milk. It is present in physiological conditions, but may in certain pathological conditions be absent, as in carcinoma, in atrophy of the mucous membrane, and in catarrhal conditions of the stomach. It may be separated from other constituents of the gastric juice in a fairly, but not perfectly pure state. It does not give the proteid reactions. By heating rennin solutions it is destroyed. This occurs more easily in acid than in alkaline solutions and enables one to prepare a rennin free pepsin solution. Hammarsten has proved that in neutral solutions obtained from certain animals, the rennin is not present but is immediately formed upon the addition of an acid. It is therefore probable that the zymogen and not the enzyme is secreted, and that the HCl present in the gastric juice causes the formation of the enzyme.

Since the cells of the glands of the fundus of the stomach consist of two kinds of cells, central and border, it was supposed that the secretions would be different from those of the pylorus. Klemensiewicz isolated a portion of the pylorus to ascertain the character of its secretion. He found it to be devoid of acid and to contain little rennin. By other methods, however, Contejean has proved that acid is secreted by the pyloric cells. He accounts for the previous results on the ground that the inflammation affected the secretions and that they were therefore abnormal and the results unreliable. The source of the HCl is undoubtedly to be found in the chlorids of the blood. But how the alkaline blood can furnish to the cells of the mucous membrane free acid has not yet been explained. It is probably due to the secreting power of the cells. The cells of the glands contain pepsin as a zymogen and not as an enzyme. The name pepsinogen has been proposed for this antecedent of pepsin. Propepsin has also been suggested.

Laboratory Work—Gastric Juice.

The silver nitrate and litmus paper test for HCl cannot be used where organic acids and chlorids may be present. In the examination of the gastric juice it is necessary to use some other method.

1. To a solution of HCl of a strength equal to that of the gastric juice,

in an evaporating dish, add 2 to 3 drops of Gunzberg's reagent and evaporate to dryness, taking care not to burn the reagent. A pinkish red color is present due to free HCl. Gunzberg's reagent is made by dissolving 1g. vanillin and 2g. of phloroglucin in 100g. alconol. HCl solution may be prepared by diluting 6 c.c. of HCl (sp. gr. 1.19) to 1 liter.

2. To 1 or 2 c.c. of the HCl solution in an evaporating dish add 2 or 3 drops of Boas' reagent. Boas' reagent is prepared by dissolving 10g. of resorcin, 3g. of cane sugar and 3 c.c. of alcohol in 100 c.c. of water. Evaporate by gentle heat to dryness,—a pink color is due to the free mineral acid. The color fades on cooling.

3. To the acid solution add one half as much of a 1 per cent solution of lactic acid and as much of a 2 per cent solution of pepsin and repeat each of the above tests. Does the presence of the organie matter prevent the reaction or interfere with its clearness?

Several other reactions are known for the detection of free mineral acids but none are better than these. Paper saturated in tropæolin will turn pink, Congo red paper turns blue, benzopurpurin turns dark brown; but organic matter interferes with all these tests.

Detection of Lactic Acid.

1. Uffelman's Test.—To 10 c.c. of a 2.5 per cent solution of carbolic acid add a drop or two of dilute ferric chlorid, forming a deep purple color; use this as a reagent. To about 3 c.c. of the solution to be tested add enough of the reagent to give the liquid a decidedly purple color, and heat. Lactic acid will leave a straw yellow color, while mineral acids leave a colorless solution. Use a 1 per cent solution of lactic acid to make the test. Notice the color of the original solution, for if it is a straw color at first, the resulting solution may be straw color and yet not indicate the presence of lactic acid.

2. To about 3 c.c. of lactic acid solution add a few drops of HCl and repeat the above test; does the presence of mineral acid interfere with the reaction? To another portion add still more HCl and ascertain about what proportion will affect the reaction by discharging the yellow straw color.

3. Try the above test, using separately alcohol, sugar solution and peptone solution. Does the presence of these substances leave a yellowish color which might be mistaken for that produced by lactic acid?

The student will probably be convinced that the above test will be unreliable in the presence of substances apt to be found in the gastric juice. The lactic acid may be separated from all these by extracting the liquid several times with ether. The ether must then be distilled off and the residue be dissolved in water and tested by Uffelman's test.

Peptic Digestion.

A fluid that will serve to perform the processes of peptic digestion may be prepared by mincing the inner layer of a hog's stomach and treating

it with twice its weight of strong glycerol, allowing it to stand several days, or by dissolving 1g. of commercial pepsin in 1 liter of a 0.25 per cent solution of HCl. The latter will be used for the following experiments:

1. To 2g. of the white of a hard boiled egg, which has been rubbed through a fine meshed sieve, add 20 c.c. of the acid pepsin solution in a test tube,—label No. 1. Boil 20 c.c. of the acid pepsin solution, cool to 40°C. and add to 2g. white of egg in a test tube,—label No. 2. In test tube No. 3, treat 2g. of white of egg with 20 c.c. of 0.25 per cent HCl solution. In test tube No. 4 treat 2g. of white of egg with 5 c.c. of pepsin solution without acid. In test tube No. 5 treat 2g. of white of egg with 5 c.c. of pepsin solution and 15 c.c. of the 0.25 per cent HCl solution.–

Place the five test tubes with contents in a beaker, and place in an incubater or water bath kept at 40°. Observe occasionally and notice the changes. In which does digestion occur most rapidly? Why is there no digestion in No. 2? What changes occur in No. 5? Explain.

2. After about an hour the contents of tube No. 1 will be found digested into an opalescent liquid. Filter, and to about 10 c.c. of the filtrate in a small beaker add 8g. of powdered ammonium sulfate. Immerse from 30 minutes to an hour in a water bath. This will saturate the liquid with the ammonium sulfate and precipitate albumose. The albumose will gather as a sticky substance at the surface. Filter through a filter saturated with $(NH_4)_2SO_4$ solution and wash the residue with saturated solution of the same.

Dissolve the residue on the filter in pure water and preserve to test for albumose.

3. Take the filtrate from last experiment containing peptone.

(a) Test a portion with potassium ferrocyanid and acetic acid test. Do peptones respond to these tests? Do other proteids?

(b) Test a portion by biuret test in cold. Peptones give this test in cold.

(c) Test a portion with tannic acid.

4. Take the albumose solution made in experiment 2 above and test a portion by boiling. Does it coagulate? Does albumose coagulate by heat?

(b) Exactly neutralize the remainder by NaOH. Test a portion by nitric acid test.

(c) Test another portion by potassium ferrocyanid and acetic acid.

(d) Test another portion with tannic acid.

If the tube had remained from 3 to 5 days in digestion, very little albumose would have been found. Why?

If tubes 2 and 3 remain several days and are then observed, a solution is seen; testing shows neither albumose or peptones, but syntonin. Why?

PANCREATIC SECRETION.

The secretion of the pancreas is usually secured for analysis and experiment by fistulæ, made by inserting a cannula in the pancreatic duct of animals. It is probable that the inflammation so produced renders the fluid somewhat abnormal; but it is as nearly normal as can be obtained by any method.

The pancreatic juice obtained by a temporary fistula has a specific gravity of 1.030, while that of a permanent one has a specificic gravity of 1.010.

The pancreatic secretion is somewhat viscid fluid resembling saliva. It has a saltish taste and an alkaline reaction. Analysis by different chemists vary somewhat, but the pancreatic juice of a dog has approximately 900 parts of water, 90 parts organic matter and 10 parts inorganic salts per 1000 parts. The organic matter is chiefly proteids and ferments. The more abundant the flow the less the amount of solid matter. Sodium chlorid constitutes about seven tenths of the salts. Potassium chlorid, sodium carbonate, sodium, calcium and magnesium phosphates and traces of iron are also found. The alkaline reaction is due to the carbonate and phosphate of sodium.

The pancreatic secretion is coagulated either by cooling to 0°C., or by heating to 75°C. Alcohol, concentrated acids, tannic acid and mineral salts cause a precipitate. The precipitate by alcohol carries the ferments down with it.

The pancreatic juice readily undergoes putrefaction. If some of the secretion has been allowed to stand for a time and is then treated with chlorin or bromin water a red color is produced, whereas fresh pancreatic juice gives no color. Trytophan, a decomposition product, causes the red color. A short time afterward no color is given, as the decomposition goes on and new compounds are formed.

The work of the pancreas is not only to produce the enzymes, but it has an important action in the transformation of dextrose in animal bodies. When the pancreas of a dog has been removed a large amount of sugar at once appears in the urine. Several theories to account for this fact have been proposed, none of which are entirely satisfactory. But it is generally admitted that directly or indirectly the change of grape sugar into other substances is regulated by the pancreas.

The pancreatic gland contains globulin, nucleoproteid, albumin, nuklein, leucin ($C_6H_{13}NO_2$), xanthin ($C_5H_4N_4O_2$), hypoxanthin ($C_5H_4N_4O$), guanin ($C_5H_5N_5O$), adenin ($C_5H_5N_5$), inosite ($C_6H_{12}O_6$), lactic acid ($C_3H_6O_3$), volatile fatty acids, fat and mineral substances.

There are at least three enzymes; trypsin, which acts on proteids, amylopsin, which acts on starch, and steapsin or pialyn, which decomposes fats into free fatty acids and glycerol. Claude Bernard believed that a fourth ferment was present which emulsified the fats, but since the presence of

fatty acids due to the decomposition of fats will emulsify fat, the presence of such an enzyme is doubtful. Some authors believe a ferment which coagulates milk to be present.

Trypsin is not contained in the fresh pancreas but its zymogen only exists there. The trypsin is readily formed upon standing or by treating with acids. The zymogen is soluble in glycerol, and this substance is usually used to prepare extracts for experiments. Trypsin is formed by the splitting up of the zymogen, which occurs in watery solutions, in acid solutions and in solutions of neutral alkaline salts. Trypsin carries on its proteolytic activity in an alkaline, neutral or slightly acid medium. Its greatest activity is shown in a fluid whose alkalinity is equal to a 1 per cent solution of sodium carbonate. The activity of trypsin increases with the temperature up to 60°C and then rapidly falls, all action ceasing between 75°C and 80°C. When fibrin is digested there is no preliminary swelling of fibrin, but the outside is at once eroded and the material is gradually digested.

The digestion of proteids by trypsin is carried further than by pepsin. A globulin may be obtained as an intermediate product. Albumoses, peptones, leucin, tyrosin, aspartic acid ($C_4H_7NO_4$), lysatin ($C_6H_{11}N_3O$), lysin ($C_6H_{14}N_2O_2$), ammonia and trytophan (proteinochromogen) are produced by pancreatic digestion. Putrefaction must be prevented or other substances will be produced.

Amylopsin is a diastasic enzyme which causes starch to pass through nearly, if not exactly, the same changes which the ptyalin of the saliva causes. Maltose, dextrins, iso-maltose and a very little dextrose are the products in the case of pancreatic digestion, but it is doubtful whether saliva ever produces dextrose.

This ferment acts very rapidly and with great power. The rapidity increases with increase of temperature to 30°C. From 30° to 45° it remains unchanged. Above 45°C there is a decrease in rapidity. A large amount of the ferment converts a small amount of starch into dextrin and sugar almost instantly. Smaller amounts in proportion act less rapidly. Roberts estimated that pancreatic diastase is able to transform into sugar and dextrin, 40,000 times its own weight of starch. This ferment does not appear in children under a month old

Steapsin is the enzyme which acts upon the fat. The fat is broken up into fatty acids and glycerol. The fatty acids unite readily with the sodium carbonate of the pancreatic juice and form soap which emulsifies some of the remaining fat. The emulsion will occur when the slightest trace of acid is present. It is probable that the proteids have some influence on the emulsifying of fat. Some have supposed that a fourth enzyme was present for this work, but that is not generally accepted.

Laboratory Work—Pancreatic Digestion.

Proteolytic action of trypsin.

1. Cut into a pulp the pancreas of a recently killed animal and take

about 5 c. c. for this experiment. Place 5g. of fresh fibrin, (or an equal amount of the finely divided white of a hard boiled egg) into a small Erlemeyer flask, add 20 c. c. of 1–25 chloroform water and then add the 5 c. c. of the pancreatic pulp.

Take out about ¼ of the mixture and render decidedly acid by a few drops of HCl. Place in a covered beaker or test tube and keep at a temperature of 40°C. Render the remainder alkaline by a few drops of a sodium carbonate solution and keep at a temperature of 40°C for 2 or 3 days. Observe that the acid solution does not digest. After half an hour test a portion of the alkaline solution for albumoses. After an hour test for peptones. After several hours a disagreeable odor, due to indol (C_8H_7N) appears. This is not a true digestive product, but due to putrefaction.

After two days acidify with acetic acid, boil and filter.

a. To a portion of the filtrate apply the biuret test in the cold. What is shown to be present?

b. To another portion add a few drops of bromin water and shake. If a pink or purple red color is shown, what substance does it indicate?

c. Evaporate the remainder in a watch glass to a volume of a few cubic centimeters, allow to stand over night and examine the residue for tryosin needles or leucin balls. If none are visible, concentrate more and allow to cool for a time and look again. A more complete description of leucin and tyrosin will be given later and the material obtained above may be preserved until that time.

Diastasic action of amylopsin.

1. Prepare a starch paste by treating 1g. of starch with 100 c. c. of boiling water and allow to cool. Place about 15 c. c. in each of 2 tubes. To tube No. 1, add 2 c. c. of fresh pancreatic pulp, and to tube No. 2 add the same amount of pancreatic pulp previously boiled. Place both in water bath at 40°C. At intervals of 15 minutes test a portion of each for starch, by iodin; and for sugar by Fehling's solution. Compare the results of the test with those from the action of saliva on starch paste. Which is the more rapid? Is the character of changes the same?

Fat Splitting Action of Steapsin.

The fat for this must be perfectly neutral. It may be so prepared by mixing 10 c. c. of cottonseed or olive oil with 20 c. c. of water made alkaline with some Na_2CO_3 solution. Now add an equal volume of ether and shake until the fat dissolves. Place in a separatory funnel and draw off the water. Wash with fresh water and draw off again. Evaporate the ether over hot water. Avoid a flame while evaporating. The neutral fat remains.

1. To about 2 c. c. of the neutral fat in a test tube No. 1 add about 5 c. c. of the fresh pancreatic pulp.

b. To about 2 c. c. of the neutral fat in test tube No. 2 add about 5 c. c. of the boiled and remacerated pancreatic pulp.

Place in each tube a few drops of blue litmus and if not already alkaline, render so by a drop or two of Na_2CO_3 solution. Shake and place in incubator at 40°C. over night. Observe the condition of the fat in each tube. Observe the reaction. Why is the one in which was placed fresh pulp, acid, while the other is alkaline? Caution! If left too long acids may form in No. 2 by putrefactive changes, and will then give the following reactions similar to No. 1.

To a portion taken from each of the two tubes add about 1 c. c. of a 2 per cent (Na_2CO_3) solution, and shake vigorously. Why do the contents of tube No. 1 cause emulsification of the remaining fat? Why does a similar treatment of No. 2 not produce a permanent emulsion?

LEUCIN $C_6H_{13}NO_2$.

The structural formula of leucin is $(CH_3)_2CH.CH_2CH(NH_2).CO_2H$, and the corresponding name would be alpha-amido-isobutyl-acetic acid.

Leucin is a cleavage product in the decomposition of proteids, gelatin aud horn. It has also been found as a normal constituent of the pancreas, spleen, lymphatic glands, salivary glands, the thyroid, thymus, liver, lung and testicle.

It is formed as a result of bacterial decomposition and in pus, diseased blood and decomposing epidermis. The peculiar odor from decomposing epidermis between the toes is due to decomposition products of leucin. It has been found in plants, showing that it may result from decomposition of vegetable proteids. In pathological conditions leucin has been found together with tyrosin in urine in acute yellow atrophy of of the liver, in cases of phosphorus poisoning and in cases of cirrhosis, in the alvine dejecta of cholera patients, in certain dropsical exudations, and in the sputum in cases of pulmonary gangrene. (Gamgee.)

When pure, leucin occurs in glistening, white plates, but as usually found it is in waxy, yellowish balls, which often show faint radial markings. It is readily soluble in hot water, somewhat less so in cold, it is soluble in acids and alkalis, but difficultly soluble in alcohol. It forms salts with both acids and bases. The acid and alkaline solutions are dextro-rotary, but isomeric forms seem to exist, some of which are inactive and some dextro-rotary. Leucin forms compounds with acids and also with bases.

Laboratory Work—Leucin.

1. When heated to 170°C. leucin volatilizes and condenses in rosettes of fine, plate-like crystals. At a still higher heat it breaks up into amylamin [$C_5H_{11}(NH_2)$] and CO_2. Try this experiment.

2. Place a quantity of leucin the size of a pin head on a drop of water on a glass slide. Mix it up with the water and observe its action. Cover with a cover glass and examine. Sketch the crystals and preserve the sketch for reference. Dissolve the crystals by warming, set aside to cool, and sketch the crystals which form.

3. Add to a qnantity of leucin the size of a small pea, a few c. c. of urine in a watch glass and heat until it dissolves. Concentrate to one-third the volume, cover with beaker and set aside over night. Examine with microscope—observe the characteristic waxy, yellowish leucin balls.

4. Place an amount of leucin the size of a grain of wheat on a piece of platinum foil; add two or three drops of nitric acid and evaporate to dryness. A colorless residue will remain. Now add one or two drops of NaOH solution and heat gently—an oily drop is found. This is called Scherer's test.

5. To test for leucin in the urine it is necessary to precipitate the urine with basic lead acetate and filter. Remove excess of lead from filtrate with H_2S. Evaporate to a very small quantity and set aside to crystallize. Examine with a microscope. If leucin is present, it may be removed by warm alcohol. Tyrosin may be detected in the same manner.

TYROSIN—$C_9H_{11}NO_3$.

Tyrosin has the euphonious title of para-oxy-phenyl-alpha-amido- propionic acid, with the formula, $C_6H_4OHCH_2CHNH_2COOH$.

It is probably never found in healthy tissue, but is always the result of morbid or putrefactive changes, or of the digestive action of trypsin. It may also be produced by the action of acids or proteids or horn, but not from gelatin. It is found in the urine in acute yellow atrophy of the liver and occasionally in phosphorus poisoning. It is also claimed that it has been found in cirrhosis of the liver, in severe cases of typhoid fever and in small pox. Simon states that he has been unable to find tyrosin in any case of typhoid fever or small pox.

Tyrosin forms in delicate, silky needles, often grouped in bundles. These needles melt at 235° C. From very impure solutions it separates in balls much like those of leucin. Tyrosin is soluble in 1900 parts water at ordinary temperatures, and in 150 parts boiling water. It is insoluble in alcohol and ether, but it dissolves in alkalis readily, and in acids it forms soluble compounds.

Laboratory Work—Tyrosin.

1. Dissolve on a glass slide and examine as in experiment 2 under leucin.

2. To 1 c.c. water in a test tube add a small amount of tyrosin and dissolve. Then add a few drops of Millon's reagent, and heat the liquid to boiling. It colors rose red, aud later becomes dark red. This reaction shows the oxyphenyl group.

3. Place some crystals of tyrosin on platinum foil, add nitric acid and warm. The tyrosin becomes bright orange yellow and dissolves. Add a few drops of NaOH solution and a deep reddish yellow solution results. This on evaporation leaves an intense blackish brown residue.

4. To a boiling aqueous solution of tyrosin add some dilute acetic acid

and then a little sodium nitrite solution, drop by drop; a beautiful red color results.

Intestinal Digestion as Affected by Bacteria.

The bacteria which thrive in the presence of the pancreatic fluids set up a fermentation which causes carbohydrates to yield lactic acid, carbonic acid, hydrogen and butyric acids. This is the chief cause of gases in the intestines. Vegetable foods increase the amounts.

The bacterial action also continues the work of the steapsin on fats and produces lower acids such as valeric and butyric, thus increasing the acid character of the contents of the lower intestines. These organic acids do not stop the action of pancreatic juice.

Proteids are broken up by bacterial action into leucin, tyrosin and later into skatol (C_9H_9N), indol (C_8H_7N), and phenol (C_6H_6O). Cholin, a poisonous decomposition product of lecithin, by bacterial action, yields carbonic oxid, methane and ammonia. These putrefactive changes in excess are harmful, but probably aid in preventing the absorption of poisonous products when not in excess.

Indol, skatol and phenol may be found in the vomit of an individual suffering from obstruction of the intestines. Indol may be found in normal urine as indoxyl sulfuric acid or indican. This is greatly increased in intestinal obstruction. Indol may be obtained from stercoraceous matter in vomit, by adding phosphoric acid and distilling. Extract the distillate with ether, separate and evaporate ether. To test it, dissolve in alcohol and put a piece of pine stick previously moistened with HCl. It will assume a red color.

Its alcoholic solution turns red when fuming nitric acid is added, and its aqueous yields a red precipitate with the same reagent. Phenol may be identified by tests previously given.

INTESTINAL JUICE—(Succus Entericus).

The difficulty of securing this digestive fluid in a normal condition is so great that little value is placed on analysis. It is highly probable that it is not alike in different portions of the intestinal canal. In the duodenum and first part of the jejunum, where the glands of Brunner are mingled with those of Lieberkuhn, it probably has a very different action from that where one set of glands alone furnish the fluid. These fluids are alkaline, do not have any proteolytic activity, nor do they act upon fats, except by the presence of free fatty acids. There is, however, an enzyme which has an inverting power on cane sugar. This is supposed to be like the invertin of yeast, as its action is similar. The mucous membrane of the lining has this inverting power to a greater degree than the intestinal juices. For this reason the membrane is supposed to contain a zymogen of the inverting ferment. It has been a subject for explanation that an inverting substance should be so generously supplied, where in a healthy human being little

cane sugar ever reaches; but the fact that maltose seems to be transformed during absorption into dextrose, leads to the view that this is the chief work of the invertin.

BILE.

The bile is, like the pancreatic juice, obtained from a temporary or permanent fistula. It is, in either case, not perfectly normal. It is a viscid fluid of alkaline reaction and a bitter taste. It contains mucin from the cell lining the gall bladder and bile duct. Its specific gravity varies from 1.01 to 1.04. The color of bile varies in different animals; in human bile it is yellowish, greenish or reddish brown. At death the bile bladder is full of bile more concentrated than that obtained by fistula. The bile is continuously secreted, but is poured into the duodenum more rapidly when food enters it. Another period of more rapid secretion occurs a few hours later. This second increase seems to be due to the arrival in the liver of the products of digestion. The quantity of bile secreted varies greatly. From 0.6 to 1 liter has been obtained from a fistula, and it is probable that in normal conditions half a liter is secreted in 24 hours.

Since the bile flows from the bile bladder under very little pressure, a slight obstruction may cause its retention and it will then be absorbed into the blood and be excreted in the urine. The amount so absorbed may be large enough to produce jaundice. The bile in such a case is not, however, re-absorbed by the blood vessels, but by the lymphatics of the liver and is carried through the thoracic duct to the blood. Bile contains certain salt of bile acids, bile pigments, lecithin, cholesterin, soap, neutral fat, urea, salts of calcium, magnesium, sodium, potassium, iron and copper. There is in bile a mucoid nucleo albumin, but it is evidently not the product of the action of the liver cells.

The Bile Acids are usually present as sodium salts. These acids are a glycocholic and a taurocholic. There are several glycocholic acids and taurocholic acids. In the bile of some animals both kinds of acids are present, and in others only one kind. Upon cleavage, glycocholic acid yields glycocholl and cholic acid, while taurocholic acid yields taurin and cholic acid.

Glycocholic acid — Glycocholl — Cholic acid

$$C_{26}H_{43}NO_6 + H_2O = CH_2NH_2CO_2H + C_{24}H_{40}O_5$$

Taurocholic acid — Taurin — Cholic acid

$$C_{26}H_{45}NSO_7 + H_2O = NH_2.CH_2.CH_2.SO_2OH \quad C_{24}H_{40}O_5$$

The difference between glycocholic and taurocholic acids, easily remembered, is that taurocholic acid contains sulfur, the other does not. The bile acids may be precipitated from their solutions in water or alcohol, by ether, in which case they occur in fine, white needles. The bile acids and their salts are dextro-rotary. The bile acids are undoubtedly elaborated in the liver from proteid material furnished by the blood.

The Two Bile Pigments in normal human bile are bilirubin ($C_{32}H_{36}N_4O_6$) and biliverdin ($C_{32}H_{36}N_4O_8$). The former is most abundant in human bile and gives to it the yellowish red color, while the latter imparts the green color to ox bile. Bilirubin is identical with haematoidin, which is found in old blood stains. This fact points to haemoglobin as the origin of bilirubin and it may be shown that bile pigments may be formed elsewhere than in the liver cells. Bilifuscin, biliprasin and bilicyanin have been found in bile stones and bile which has undergone chemical change.

The bile pigments are insoluble in water, nearly so in ether and alcohol; chloroform dissolves bilirubin, but does not dissolve biliverdin. Both pigments are soluble in alkalis, insoluble in acids and yield with calcium and other metals compounds insoluble in water. Calcium bilirubin is found in bile stones. There is in the mucin of the bile obtained from the mucous glands of the bile sack and duct a nucleo albumin, which gives to the bile its viscid character. A nucleo albumin is similar to mucin, but yields on splitting up a proteid and phosphorus-containing nuclein, instead of a proteid and a carbohydrate which mucin yields.

Cholesterin ($C_{27}H_{45}OH$ or $C_{26}H_{43}OH$), a monatomic alcohol, is present in human bile from 0.5 to 3.5 per thousand. While cholesterin is insoluble in water it is soluble in a mixture of salts of bile acids, soaps and fats, and it is by these that it is held in solution in the bile. Cholesterin is an important constituent of nerve tissue and seems to be excreted from the blood by the liver. It is found in the white substance of Schwann as well as in white blood corpuscles. Cholesterin is found in gall stones. Indeed, some gall stones are chiefly composed of cholesterin. A description of cholesterin crystals and tests will be given under the analysis of gall stones. Lecithin seems also to be present in the bile as an excretory product, as it is more abundant there than elsewhere in the body. The function of excreting lecithin and cholesterin is not fully established, but is not improbable. Minute traces of a diastasic ferment have been found in the human bile, but in quantities too small to be of consequence. The inorganic salts are sodium chlorid, sodium phosphate and sodium carbonate, a small amount of potassium chlorid, a little calcium or magnesium phosphate and traces of copper, iron and manganese. The action of the bile as a digestive fluid has been the subject of much discussion. It has no ferment in sufficient quantity to affect digestive processes, and yet it is undoubtedly a digestive, as well as an excretory fluid. The bile exerts no material diastasic action and no direct proteolytic action. It does, however, aid in neutralizing the acidity of the gastric juice and thus prepare for pancreatic digestion. The bile acids probably precipitate native albumins and thereby hasten their digestion by the pancreatic fluids. The bile acids are also precipitated and probably dissolved gradually and re-absorbed. After reabsorption they find their way back to the liver, instead of passing out with the faeces.

The bile acids will not alone form a permanent emulsion of fats, but

when mixed with the fatty acids produced by the action of steapsin, a permanent emulsion of fats is at once formed. Bile is said to exercise an antiseptic action on the contents of the intestines. Bile itself will readily undergo putrefaction, but free taurocholic acid is a strong antiseptic and it is probable that the acid of the chyme precipitates this acid in a free state, and that the putrefactive changes are thereby retarded. Bile is a laxative. Animals having a biliary fistula are constipated. Constipation may usually be relieved by gently pressing the bile into the intestines with the fingers. This mode of treatment is undoubtedly preferable to the cholagogues which are so apt to injure elsewhere more than they help at this point.

Laboratory Work—Bile.

1. Dilute some bile with 2 volumes of water, heat and test with litmus paper. The reaction should be alkaline.

2. Dilute some bile with 2 volumes of water, add acetic acid and notice the precipitate of mucin or nucleo albumin. (This is not so noticeable in ox bile as in human bile.) Preserve for following experiment.

3. Filter the precipitate of the preceding experiment, and test for proteids by biuret test. Are proteids present?

4. Mix about 5g. of animal charcoal with 20 c.c. of bile and evaporate to complete dryness. Transfer to an Erlenmeyer flask with condensing tube, add about 30 c.c. absolute alcohol and boil on a water bath for half an hour. Filter into a dry flask, cool and add ether until a permanent precipitate forms. Cork and set in a cool place over night. Filter the crystals off in a cool place. Examine, dissolve in water and test by

Pettenkoffer's Test as follows: To the solution containing bile acids add 1 c.c. of H_2SO_4 slowly, so that it will flow down the side of the tube and form a layer at the bottom. Add a drop or two of cane sugar solution, or 3 or 4 small crystals of cane sugar and agitate gently. Avoid mixing the sulfuric acid with the liquid above, but mix the sugar with the liquid down to the surface of the acid. A reddish purple or magenta color will appear. If too much sugar is added a brownish red color is produced. The reaction does not occur immediately, but after standing a few moments. A similar test, using one drop of furfurol solution instead of sugar may be tried.

5. Urine containing one part of bile to 100, also one part to 500 should be tested by Pettenkoffer's test. Each test should be compared to a similar test of normal urine.

6. A 1 per cent aqueous solution of furfurol may be used instead of sugar, as the sugar and acid form fururol. It is used in the same manner as the sugar in Pettenkoffer's test, and one or two drops is usually sufficient. Try the bile solutions as above with this test.

Tests for Bile Pigments.

1. Gmelin's Test— in a small evaporating dish place some bile solution or suspected urine, and add a drop or two of fuming nitric acid; a play

of colors, green, blue and violet results. In urine tests the green color is more important.

a. A modification of this test may be used as follows: Filter the suspected urine and place a drop of fuming nitric acid on the filter. The rings of color form about the drop, changing through the colors, green, blue and purple.

b. Another form of this test is to pour about 2 c. c. of fuming nitric acid in a test tube and gently let an equal quantity of urine containing bile, flow in upon it. The play of colors as above is seen at the surface.

2. Iodin Test. To about 4 c. c. of the suspected urine add 2 or 3 c. c, of a 1-10 solution of iodin in alcohol. In adding the iodin pour it gently down the side of the tube so as not to mix. At the junction of the two solutions a bright green ring forms. This test is recommended highly by some.

GALL STONES.

Calculi are usually composed pf cholesterin, and bilirubin, sometimes of nearly pure cholesterin. They vary in size from that of a grain of rice to an inch in diameter. There are sometimes few, and sometimes many. Over 7000 have been found in one gall bladder. Their color varies from a light gray to a black. The color depends upon the amount of bilirubin. This pigment is present, not in a pure state, but as the calcium salt. The structure is usually that of concentric layers around a nucleus. The calculi found in the gall bladder of cattle are usually composed of calcium bilirubin. Gall stones of calcium carbonate and phosphate, sometimes found in animals, are very rarely found in man.

Laboratory Work—Gall Stones.

1. Pulverize and warm gently in a mixture of alcohol and ether. Filter and evaporate the filtrate to dryness. The cholesterin crystals may be examined by the microscope. To purify them, dissolve in alcohol and recrystallize. Preserve for further examination.

2. To the residue on the filter add HCl; if calcium carbonate is present effervescence will occur. If a residue, insoluble in HCl, remains, examine for bile pigments. Evaporate that which dissolved in HCl, to dryness, ignite, take up in a little HCl and add ammonia; if a blue color is shown copper was present.

3. Place some of the cholesterin crystals on a glass slide and cover with cover glass. Allow a drop of dilute sulfuric acid to flow under the cover glass. The cholesterin crystals show a bright red on edges, changing to blue. Warmth will hasten the reaction.

4. To some cholesterin crystals on a slide add a drop of dilute sulfuric acid and then a drop of iodin solution. The crystals turn violet, bluish green and then blue.

5. In a perfectly dry test tube dissolve a few crystals in a little chloroform, add an equal volume of sulfuric acid and shake. A blood red color appears which changes to cherry red and purple.

To examine urine for cholesterin it is necessary to extract with ether, separate and evaporate slowly. The crystals may then be examined as above.

BLOOD.

A description of the physical and physiological properties is given in connection with the course in Physiology and Histology. In this department the examination of the blood by analytical processes and by the spectroscope, haemometer and haemocytometer will be undertaken, leaving to other departments a fuller discussion of blood in all its relations.

Spectroscopic Examination of Blood.

The spectroscope consists of three tubes mounted on a stand and containing a prism of glass so placed at the center that light received through one of the tubes is refracted into the other and received by the eye placed at the eye piece of the telescope, which occupies one of the tubes. At the end of the tube at which the light enters is a narrow slit capable of adjustment. The third tube contains a scale which is reflected on the prism by placing a lamp opposite its end. This scale enables any lines on the spectrum to be definitely located. Place a fish tail burner opposite the slit and a continuous spectrum is observed. Place a platinum wire with a borax bead on it in the flame of the burner, and the location of the sodium line may readily be observed. Note its position on the scale. Then place the glass cell containing a dilute blood solution (1:50) between the flame and the slit of the spectroscope. Two dark bands near each other' and the left one immediately to the right of the sodium line, may be observed. These are the absorption bands of oxyhaemoglobin.

2. To the blood in the cell add one or two drops of a solution made by dissolving two parts, ferrous sulfate and 3 parts tartaric acid in water and rendering alkaline with NH_4OH. This solution, called Stoke's solution will reduce the oxyhæmoglobin and form reduced hæmoglobin. The two bands disappear and are replaced by a single wide band of reduced hæmoglobin, the edges of which are a little farther to the left than were the outer edges of the other two bands.

3. To the contents of the cell now add a few drops of concentrated NaOH. The single absorption band of reduced hæmoglobin gives way to two bands similar to those of oxyhæmoglobin, but further to the right. These are the absorption bands of hæmochromogen. The left band is the darker of the two. Upon standing, the two bands merge into one broad one.

4. Empty and rinse the cell, then fill it ⅓ full of a solution of blood

1 part to 15. Observe the spectrum. It will be found to be entirely dark to the right of the sodium line. Dilute by adding water and gently stirring with a glass rod, until the spectrum of oxyhæmoglobin as first seen appears. The student will observe that the width and darkness of the bands depend upon the concentration. If the dilution is carried still further, the bands become still narrower and fainter, the right one fading out first.

5. To the blood solution now add 1 or 2 drops of concentrated potassius ferricyanid solution. The color of the liquid changes to a brown. Observe the spectrum. There should be a dark band in the red and two dark bands to the right of the sodium line. If it is too concentrated, dilute. This is the spectrum of methæmoglobin.

6. To about 10 c.c. of concentrated H_2SO_4 in a test tube add about 5 drops of blood. Shake thoroughly after the addition of each drop of blood and keep the tube cool. What color has the solution? Examine the spectrum. This spectrum is produced by hæmatoporphyrin ($C_{16}H_{18}N_2O_3$), and shows a band to the left and a dark band to the right of the left band of oxyhæmoglobin.

7. Pass a current of CO through a dilute solution of blood (1-50). Examine the spectrum. Two bands similar to those of oxyhæmoglobin. but slightly farther to the right, appear. Add a few drops of fresh, strong potassium ferricyanid. It is not changed to methæmoglobin as the oxyhæmoglobin was in experiment 5. This shows that CO-hæmoglobin is so stable as to be difficult to restore or change when the blood is once poisoned by this gas.

General Tests.

1. Place some defibrinated blood on a piece of red litmus paper; wash it off after a few seconds. If not too much blood clings to the paper, the blue color of an alkaline reaction will be discernible. A glazed litmus paper is the best for this work.

2. Prepare a small plate of plaster of Paris, dry it and then saturate it with a neutral litmus solution. A drop of blood may be placed upon it, left for a short time and then washed away. The blue reaction will be shown.

3. Take about 4 c.c. of water in a test tube, add two drops of blood and mix. Then add tincture of guiac until the liquid becomes cloudy. Pour a layer of old turpentine on the top and allow to stand. A blue color develops at the line of contact. This reaction fails unless the turpentine is old. Hydrogen per oxid may be used to good advantage instead of turpentine.

4. To about 2 c.c. of blood add an equal amount of hydrogen per oxid solution. The oxygen of the oxyhæmoglobin will be liberated and cause a violent effervescence. The hydrogen per oxid causes the hæmo globin to give up its oxygen by catalytic action.

5. To about 2 c.c. of diluted blood (1–5) add 2 c.c. of ether. Shake until the appearance of the blood is changed. This is called laky blood. The hæmoglobin dissolves in the ether.

6. Heat for half an hour in a flask provided with a condensing tube, about 10 c.c. of glacial acetic acid. Then add gradually and by constant stirring 3 c.c. of defibrinated blood. Heat on the water bath for half an hour. Transfer to a small beaker and set aside over night. Examine for hæmin crystals. Hæmin crystals are very small, oblique prisms and plates of a reddish brown color.

7. Scrape dry blood stains from any object that may have them. Pulverize and moisten with dilute salt solution. Place on a slide with a drop of glacial acetic acid and cover with cover-glass. Heat nearly to boiling for some time. Allow to cool and examine for hæmin crystals. In this way blood stains may be positively identified. Spectroscopic and microscopic examination may in legal cases supplement the hæmin crystal test. From a clot or an old blood stain may be soaked in a very little salt solution and tested as above.

8. From about 20 c.c. of defibrinated blood diluted with 200 c.c. of water is a beaker, precipitate the proteids and corpuscles by very slightly acidulting with acetic acid and boiling. Filter, concentrate by evaporation, to about 20 c.c. and filter again. Render alkaline and test some of this with Fehling's solution for sugar.

9. Acidulate with nitric acid another portion of the fluid obtained in experiment 8 and test with silver nitrate for NaCl.

10. To a third portion add ammonia and magnesia mixture to test for phosphates.

11. Evaporate the balance to dryness and examine with the microscope for NaCl crystals.

THE NUMBER OF RED CORPUSCLES IN BLOOD.

Thomas' hæmacytometer may be used to determine the number of blood corpuscles. In the normal blood of man there are about 5,000,000 blood corpuscles in a cubic millimeter. There is a somewhat greater number in the blood of men than in the blood of women. In certain pathological conditions, called anaemia, the blood is deficient in red corpuscles.

Thomas' hæmacytometer consists of a mixing pipette and a glass slide with a small cell in the center, which has the bottom ruled into given spaces. A drop of blood obtained by pressing a shielded needle through the skin is drawn up into the pipette to the line marked 1. Then a solution of sodium sulfate with a specific gravity of 1.015 is drawn into the pipette to a line marked 101. Since the 1 volume in the capillary tube may be expelled later unmixed, the mixture is now 1 per cent blood. By shaking the pipette the fluids become equally mixed and a drop, after expelling the Na_2SO_4 solution from the capillary tube, may be placed in the cell. A cover glass laid upon it will spread the blood uniformily over the bottom of the cell. Each square covers 1-400 of a millimeter. The slide is placed under

a microscope and the corpuscles counted. The thickness of the blood layer is 1-10 of a millimeter. An average number obtained by counting 10 squares may be multiplied by 400x100, and the number of red corpuscles per cubic millimeter will be obtained.

THE COLORING MATTER OF THE BLOOD.

Whether the coloring matter of the blood is haemoglobin, or whether haemoglobin is a decomposition product is a disputed question; but no antecedent of haemoglobin has yet been separated.

Haemoglobin is found in the red blood corpuscles and in striped muscle. It is composed of carbon, hydrogen, oxygen, nitrogen, iron and sulfur. Upon decomposition it yields albumin, small quantities of volatile fats and a coloring matter called haemochromogen. The haemochromogen upon oxidation yields haematin. The haemoglobin forms, while the blood is in the lungs, a loose compound with oxygen called oxyhaemoglobin, but when the blood reaches the tissue where oxygen is deficient, it is given off and is used by the tissues. The reduced haemoglobin is carried around in the circulation until the lungs are again reached when it repeats the process of absorbing oxygen. The blood in the arteries is rich in oxyhaemoglobin while that in the veins contains more reduced haemoglobin.

Both haemoglobin and oxyhaemoglobin may be obtained in crystalline form, the latter more easily than the former. The crystals obtained from the blood of different animals differ in shape. There is considerable variation in the amount of haemoglobin in the blood, it averages about 14 per cent. Various compounds and transformation products are yielded by haemoglobin, such as carbon monoxid-haemoglobin, methaemoglobin, pseudo-haemoglobin, haemochromogen, etc.

THE AMOUNT OF HAEMOGLOBIN.

Sometimes the lack of color in the blood is not due to a deficiency in the number of red corpuscles, but to a deficiency of haemoglobin. By comparing the redness of the blood with a prepared scale of redness the per cent of haemoglobin may be closely approximated. This may be done with Fleischl's haemometer. This instrument consists of a glass cell with a partition. In one side distilled water is placed; in the other a given quantity of a dilute solution of blood. By moving a glass wedge containing a gradually increasing amount of red coloring matter under the cell until the blood solution and glass are of the same intensity, a scale attached to the wedge gives the per cent of haemoglobin. The following instructions should be observed in making this test:

The instrument should be adjusted in a dark, cool room with a lamp or gas light opposite the mirror, so that the light can be reflected up through

the cell. The colored wedge should be placed under the cell. One portion of the cell over the wedge should be filled with distilled water by means of a pipette, so that its surface is exactly even with the edge of the cell. The other portion of the cell into which the blood is to be placed, is filled about one fifth full of water and the pipette so placed that when the blood is measured, it can be conveniently washed into the cell.

The measuring pipette may be slightly smeared with oil on the outside, to avoid the adhesion of the blood. A needle is used to pierce the skin of the ball of the finger, a drop pressed out and the measuring pipette filled by passing it horizontally into the drop. The outside may be cleaned by a cloth smeared lightly with oil, care being taken to have the blood exactly even with the ends of the pipette. Place the pipette in a horizontal position in that portion of the cell partly filled with water and allow the wire handle to rest on the edge of the cell opposite the partition. Move the cell gently through the water to wash out the blood and to mix it evenly through the water. The measurement and mixing of the blood with the water must be done so quickly that it does not coagulate. When the greater part of the blood is washed out of the pipette, place it in a vertical position over the center of the side containing the blood and allow water from a pipette to drop on the end until every trace of blood is washed into the cell. Great care should be taken that no drops spatter out. The blood is then mixed thoroughly with the water by means of a fine wire. The part of the cell containing the blood is then filled until the surface is exactly even. Avoid either a concave or a convex surface. Carefully mix with the fine wire.

Place the eye directly over the cell and move the glass wedge until the color of the wedge is precisely like the color of the dilute blood solution. The light from the reflector should be stronger when the color of the blood solution is deeper. The eye and the surface of the fluid should be protected from the light of the lamp, so that all the light may come through the wedge and fluids. The eye should not be tired by looking too steadily but should look at other objects occasionally. When the position of the wedge is reached in which an equal color is seen in both cells the reading of the scale gives the per cent of haemoglobin.

BLOOD PLASMA.

Blood plasma is alkaline, yellowish in tint and has a specific gravity varying from 1.027 to 1.032. Besides extractive matters and inorganic salts it contains fibrinogen, serum albumin and serum globulin. The student should review what is given in these notes concerning these bodies under "Proteids."

Blood serum is the fluid obtained after blood has been allowed to clot, and contains the same substances as blood plasma, except that fibrin fer-

ment is found in considerable abundance instead of fibrinogen. This fibrin ferment does not seem to exist in the blood in appreciable quantities until it is shed, or until some foreign substance is introduced into a vein or artery. Blood clots readily when shed into any except an oiled vessel and then it may be stirred by an oiled glass rod without clotting. Stirring with a glass rod which has not been oiled will cause clots to form on the rod. We may conclude that adhesion to a surface causes clotting, whether within or without the blood vessels. The fibrin ferment forms in the blood when the clotting occurs, but what its antecedent may be is not known. Indeed, the change of fibrinogen to fibrin is not understood. Fibrin ferment will cause coagulation of serous and hydrocele fluids.

MILK.

The average composition of cow's milk as a result of many analyses is as follows: Water 87.4 per cent. fat 3.5 per cent sugar 4.5 per cent, proteids 3.9 per cent, salts 0.7 per cent. The total solids are given as 12.6 per cent. The proteids are casein, globulin and albumin. The sugar is lactose, commonly called milk sugar. Recent and careful analysis of human milk gives the following: Water 87.81 per cent, fat 4.06 per cent sugar 5.26 per cent proteids 2.36 per cent salts .51 per cent. It will be noticed that human milk is richer in sugar and fats, but poorer in proteids.

The specific gravity of mixed milk from a number of cows varies from 1.029 to 1.034. The reaction is usually alkaline or amphoteric. It may be acid, and is usually so in carnivorous animals. The acid character of milk which may be observed upon standing, is due to the formation of lactic acid by fermentation of lactose. The color of milk depends upon the floating particles of fat and the casein held in solution by calcium phosphate. Fresh milk will not curdle when heated, but after lactic acid formation begins, heat at once causes a curd to form. Sterilized milk will keep sweet indefinitely. When rennet is placed in milk a coagulum soon forms, if a calcium salt is present. As stated under proteids, caseinogen is present in milk and is broken up by coagulation into two proteids. The casein resulting from the separation reunites with calcium to form the curd, and a whey-proteid remains in solution.

The scum which forms on fresh milk when heated is not a coagulated albumin, but a combination of calcium and casein. It will soon form again if removed. Casein was prepared under the subject of derived albumins. It is insoluble in water, but dissolves readily in alkalis. It may be precipitated by dilute acids, common salt or magnesium sulfate.

The globulin of milk is probably identical with the serum globulin. The albumin is closely related to serum-albumin but is not identical. It differs in coagulation, temperature, solubilities and specific rotary power. It is called lact-albumin. The percentage of the different proteids are about

as follows: Casein 3.19 per cent albumin 0.36 per cent globulin 0.15 per cent. (Schlossman.)

The fat is present in globules which vary greatly in size. The source of the fat is not positively known. It may be brought by the blood or it may result from changed protoplasm. The lactose (milk sugar) is believed to be the result of a glandular action and may be derived, as casein is supposed to be, from a nucleo-albumin of the blood. It is fermented by yeast, but reduces Fehling's solution. It may appear in the urine of women shortly before or sometimes after confinement.

Laboratory Work.—Milk.

1. Examine a drop of milk under the microscope.

2. Boil about 5 c.c. of milk and test reaction with litmus paper.

3. To the milk used in experiment 2 add one drop of dilute acetic acid and continue heating. It coagulates.

4. In each of 5 test tubes place 5 c.c. of fresh milk. To No. 1 add ¼ c.c. very dilute HCl; to No. 2 add ¼ c.c. 1 per cent solution Na_2CO_3 solution; to No. 3 add ¾ c.c. of saturated ammonium oxalate solution. Then to each one of the five add 2 drops of rennet solution and mix. Heat tube No. 5 to boiling and allow to cool to 40°C. Place the five tubes in a water bath at 40° and examine at intervals of 5 minutes. Tubes 1 and 4 soon coagulate; the others do not. The coagulum in tube 1 is similar to that found in the stomach and contains para-casein and fat. The oxalate in tube 3 removes the calcium and prevents the formation of a coagulum. Compare with the action of an oxalate on blood. If $CaCl_2$ be added to tube 3 and the heating continued, it will coagulate also.

5. To some milk in water add tincture of guiac and old turpentine. Does it not give the same test as does blood?

6. To 1 c.c. of milk add 4 c.c. of alcohol and shake. Notice the precipitation of proteids.

7. Measure out 10 c.c. of milk and dilute it with water to make 200 c.c. Add 5 c.c. of a copper sulfate solution (Fehling's No. 1) and then enough sodium hydroxid to give a voluminous precipitate. Shake and filter. Test the filtrate for sugar by Fehling's test. The sugar which causes the reaction in this case is lactose.

8. Mix equal volumes of Millon's reagent and milk in a test tube and boil. The bulky, reddish precipitate shows proteids.

LAURENT'S POLARIMETER.

This instrument is used to determine the specific rotary effect of substances on light. Or if the specific rotary power of a substance is known, the strength of a solution of that substance may be determined. The specific rotary power is shown by the degree of rotation produced by one gram

of a purified substance dissolved to form one c.c. of liquid and examined in a layer one decimeter thick.

If a = the observed rotation,
p = the weight in grams in 1 c.c. of the liquid,
l = the length of tube in decimeters,
A_D = the specific rotary power for the sodium flame,

then $A_D = \frac{a}{pl}$

For dextrose the specific rotary power is about $+53°$. Plus meaning rotation to the right and minus meaning rotation to the left. From the above formula the per cent of any sugar solution may be determined, if the specific rotary power is known.

Experiment 1. Place the instrument in a darkened room. Opposite the end containing the polarizing crystal, place a Bunsen burner and adjust a platinum wire containing some sodium salt, as borax or common salt, in the flame. A common lamp may be used. Draw out the eye piece until a clear field is visible upon looking through the instrument toward the light. Adjust the thumb screw connected to the tube containing the analyzer until the two halves of the field are of an equal shade. At the same time keep the zero mark of the circle opposite the zero mark of the vernier at the top of the circle. It is a good plan to have a tube filled with distilled water in place in the instrument when the adjustment is made. After the instrument has been adjusted remove the distilled water and place in the instrument a tube containing a solution of dextrose (1—10). In filling the tube with a solution of any kind remove the cap and the glass plate over the end of the tube. Fill the tube so that a slightly convex surface is formed and slip the glass plate on so as not to inclose any air bubbles. When the solution of dextrose is placed in the instrument, it will be found that the field is not equally illuminated. Turn the circle until the field has an equal shade. The reading indicates the amount of rotation produced by the solution. An average of several readings should be taken. As the dextrose ordinarily used is not pure, the per cent of pure dextrose may be calculated by the student, using the formula and specific rotary power for dextrose above given.

Experiment 2. Fill a tube with a 20 per cent solution of cane sugar and ascertain whether it rotates the plane of polarization to the right or to the left.

To a 10 per cent solution of cane sugar in a beaker add 10 c.c. of concentrated HCl, and warm to 70° for ten minutes. Cool and fill the tube. *Avoid getting the acid solution on the brass part of the tube.* It will now be observed that the solution rotates to the left. Since the solution was diluted by HCl the reading must now be multiplied by 1.1. The solution is called invert sugar. By this method the per cent of cane sugar can be determined even when other optically active substances are present. As this determination is not often of clinical or dietetic importance, it need not be included here.

URINALYSIS.

The analysis of urine is recognized by Osteopaths as a valuable aid in the diagnosis of disease, and the determination of its cause.

Collection.

The collection of urine for a whole day is sometimes important; when that is desired, reject the first urine excreted after rising in the morning and begin to collect from that time. Include that passed first on the following morning. In this manner one will have a correct measure of the total amount of urine passed in 24 hours. When a small quantity only is desired, it should be collected three hours after a meal.

Color.

Normal urine is described as straw yellow, but may vary widely without great pathological significance. Its chief coloring matter is urobilin. Urine in various conditions may be almost colorless, reddish yellow, pale yellow, red, reddish brown, brown or dark brown. Its color has a significance in that it indicates the character of some constituents present, and in a measure, their quantity. It cannot be depended upon without additional examination. Light colored urine is apt to contain albumen or sugar; a red color may show urates, uric acid or blood; a green color may be due to bile. Urine may be cloudy from pus, phosphates, bacteria, blood, epithelium, urates, oxalates and possibly other substances. The color of various constituents will be mentioned under the sections in which their determination is described.

Odor.

The odor may be due to decomposition going on within the body, to putrid matter from an abcess in the urinary tract, or other cause. It is important only when other tests confirm its indications. Certain medicines give a peculiar odor to urine.

Reaction.

Normal urine is ordinarily acid but may, when much vegetable food is eaten, be alkaline. Urine will sometimes give both acid and basic reactions with litmus paper; it is then called amphoteric. This condition has no pathological significance, but is due to acid and alkaline phosphates of sodium. Some hours after a hearty meal urine may become alkaline, but soon returns to its acid reaction. Urine always becomes alkaline by standing, but the change may be delayed by antiseptics such as choroform or salicylic acid.

The alkaline reaction of old urine is due to ammonium carbonate formed by the decomposition of urea. If bacteria are found in fresh urine and

it also has an alkaline reaction, there are indications that decomposition is going on in the bladder. An abundance of rod-like bacteria indicate decomposition even though the reaction be still acid. Decomposition in the bladder may be caused by the use of a dirty catheter, or by diseased conditions. The alkalinity due to ammonia does not give a permanent color to litmus paper, but passes off in a few moments, which enables one to distinguish between volatile and fixed alkalis.

Specific Gravity.

The specific gravity varies in healthy urine from 1.005 to 1.030 and may be even higher, when little water is drank, or when prespiration is free. A high specific gravity and a large quantity is usually present in diabetes mellitus and though it may be due to medicines, it usually indicates disease. A very low specific gravity is also a matter for investigation. Some change in tissues preventing the proper elimination of urea or other normal ingredients in urine is probably going on. In acute yellow atrophy of the liver, in structural diseases of the kidney and in conditions of mal-nutrition in general there is low specific gravity of urine.

Gravity Test.

The specific gravity is ascertained by placing some of the fluid to be tested in a glass cylinder. Remove any foam from the top with filter or other clean absorbing paper and place a clean, dry urinometer in the liquid. See that the urinometer floats freely and does not touch the sides of the cylinder. Place the eye on a level with the top of the liquid and read the markings on the urinometer at the lower surface of the meniscus. To correct the reading for temperature add to it one thousandth for every three degrees Centigrade which the temperature is higher than the temperature at which the instrument is graduated. Urinometers are usually graduated at 60° F., 15.5°C, or 77° F., 25°C.

Albumin.

Albumin may be temporarily found in the urine of healthy individuals, but only in very small quantities. This may occur after eating much egg albumin, or after some cause which produces alteration of blood pressure.

Albumin found frequently or in any considerable quantities is pathological.

(1.) It may indicate Bright's Disease—an organic disease of the parenchyma of the kidneys. This is rendered almost certain when casts are found. The appearance of much albumin for weeks or months renders this disease very probable.

(2.) a. When blood, blood plasma or pus is found in the urine it contains albumin. This indicates a local disease in the urinary tract.

b. In rare cases albumin may be due to an abundant mixture of the spermatic fluid.

Microscopic examination will reveal whether the cause be of this character or not.

(3.) A hyperaemic condition of the kidneys, certain alterations in the blood due to insufficient nutrition on account of retarded metabolism, heart disease or high fevers causing sudden changes in blood pressure may produce albumin in the urine.

The necessity for microscopic examination of sediment in all cases where albumin is found is apparent from the above statement.

Globulin is found in urine but its significance is the same as that of albumin and it responds to the tests given for of proteids.

Albumose is also found in urine but has no certain pathological significance.

Mucin may come from the vagina, but if from the urinary passages indicates an irritated or catarrhal condition. It is precipitated by acetic acid or a very little nitric acid, but is dissolved by excess of nitric acid.

Tests for Albumin.

(1.) Filter, if turbid, and heat a portion of the clear fluid; if a precipitate forms it may be phosphates or albumin. Add one tenth of this volume of nitric acid, this will dissolve phosphates but not albumin. Too little acid may dissolve the albumin forming acid albumin. Heat alone is insufficient, for there may be alkalis present which will form alkali-albuminates. If albumin is present it will form a cloudy precipitate. This is called the coagulation test.

(2.) Warm some nitric acid and pour it gently into the test tube containing the urine to be tested, so that it will glide along the side of the tube and sink to the bottom; a cloudy ring at the surface of the acid indicates albumin. This test is not entirely reliable as traces do not appear at once and, when cold, urates may form the ring. This test may be used in the cold but the phosphates should be precipitated and removed by heating with excess of alkalis. This is called the Heller test.

(3.) The potassium ferrocyanid and acetic acid test is regarded as one of the most reliable. To the clear urine add acetic acid until reaction is strongly acid, then add a few drops of a fresh, clear potassium ferrocyanid solution (5 per cent). If a cloud of mucin appears upon addition of acetic acid, it should be filtered off before the ferrocyanid is added. This may be used to show the presence of mucin.

This test may also be tried by adding a few c.c. of the potsssium ferrocyanid solution to half a test tube full of clear urine, and after mixing thoroughly, by the addition of a few drops of acetic acid albumin is precipitated. The ferrocyanid prevents the precipitation of mucin.

The *Mercuric Potassium Iodid* test is perhaps the most delicate. The reagent is made by dissolving 33.12 grams of pure potassium iodid in 200 c.c. of distilled water and adding 13.54 grams of powdered mercuric chlorid. Warm and stir until the red precipitate of mercuric iodid disappears, leaving a clear solution. Dilute with distilled water to about 800 c.c. and add 100 c.c. of strong acetic acid. Allow to stand over night, decant the clear liquid and dilute to 1000 c.c.

To the clear urine acidulated with acetic or hydrochloric acid add the reagent drop by drop, not to exceed five drops. A cloud of albumin will precipitate if any is present. One part in seventy-five thousand may be shown by this test. If the acid throws out a cloud of mucin it should be filtered off before the reagent is added.

The Picric Acid Test.

To the clear, suspected urine add not more than half the volume of a saturated solution of picric acid; a yellowish, flocculent precipitate indicates albumin. This test is not considered entirely reliable as urates, mucin and some other bodies may be precipitated.

To Determine the Quantity of Albumin.

(1.) The *gravimetric method* is the only one that gives strictly accurate results. This is done by precipitating the albumin with a very little acetic acid and filtering on a weighed filter, washing first with distilled water, then with alcohol and finally with ether. The filter with the dry albumin is then weighed accurately and the weight of the albumin equals the sum of the weights minus the filter.

This requires expensive balances and other apparatus, besides much time.

(2.) *Esbach's Method* is much used for clinical purposes. Esbach's solution is made by dissolving 10 g. of pure picric acid and 20 g. of pure citric acid in a liter of water. It is used as follows: Fill Esbach's albuminometer to the line marked U with urine, add reagent to line marked R; mix thoroughly, stopper and allow to stand in a perpendicular position for 24 hours. The per cent can then be read off on the scale which has been graduated experimentally and made to agree with the amount determined by gravimetric methods.

(3.) *Purdy's Method* is to precipitate with acetic acid and potassium ferrocyanid and render compact by a certain rate of centrifugal motion; it is carried out as follows: Fill the percentage tubes to the 10 c.c. mark with urine to be tested, add 3½ c.c. of 10 per cent solution of potassium ferrocyanid and 1½ c.c. of acetic acid, mix thoroughly by closing the end and inverting repeatedly. Place the tube in the centrifuge and rotate until the urine is perfectly clear. Each one tenth c.c. represents one per cent, *bulk measure*, of albumin.

Grape Sugar.

Recent research has shown that sugar is present in traces in normal urine, but not in sufficient quantities to detect by the tests in common use; while these tests readily show it, when present in sufficient amounts to be of pathological importance. The continual presence of considerable quantities of grape sugar is known only in diabetes mellitus. The urine is then usually abundant and of high specific gravity. Sugar may be present temporarily and in small amounts from several causes not of a serious nature.

If albumen is present precipitate by adding 2 or 3 drops of acetic acid, heat and filter.

Tests for Grape Sugar.

(*Moore's Test.*) Render the urine strongly alkaline with sodium hydroxid and boil. It becomes first yellowish and then brown if sugar is present. Mucin gives this reaction to a certain extent and even normal urine becomes somewhat darker. Traces of sugar will not be shown by this test.

(2) *Trommer's Test.* Render the urine strongly alkaline by the addition of as much of a sodium hydroxid solution as of urine to be tested; add a few drops (3 or 4 to begin with) of a dilute solution of copper sulfate. Upon heating a precipitate of cuprous oxid is formed which varies in color from yellow to red, according to the heat, strength of solutions and amount of sugar. The presence of yellow, reddish-brown or red indicates sugar. If no yellow precipitate appears at first boiling, add a few drops more copper sulfate. A white or greenish-white precipitate may be due to phosphates. A black precipitate does not indicate sugar.

(3) *Fehling's Test.* To 4 c. c. Fehling's solution add equal volume of water and boil. The solution should remain clear. Add ½ c. c. of urine and boil again. More than 1-10 of 1 per cent will cause the yellow color of cuprous oxid to appear. If none appears with that amount add several c. c. and boil. The yellowish precipitate turning reddish with more heat and upon standing indicates sugar. If the urine is acid render slightly alkaline before testing. A greenish-white precipitate may be formed by phosphates. Uric acid may cause a yellow coloring, and even a red precipitate, hence Boettger's or Nylander's test should be used to confirm this test and it will then be found to be the most serviceable of any given.

Fehling's solution will deteriorate when mixed and allowed to stand even for a few hours. To avoid this it may be prepared in two parts, as follows: 34.64 g. pure copper sulfate crystals dissolved in 500 c. c. distilled water makes the first part. For the second part, dissolve 175 g. of Rochelle salts (potassium sodium tartrate) in about 200 c. c. pure water and 50 g. of sodium hydroxid in 200 c. c. of water. Combine these two solutions and make up to 500 c. c. with water. Place in separate bottles having perforated rubber stoppers. Pipettes marked for equal amounts (about 2 c. c.) passing through the stoppers and provided with rubber caps will enable equal parts to be mixed at will, and yet preserve the solution indefinitely. In larger quantities equal parts may be mixed by graduated pipettes or burettes.

(3) *Haine's Test.* Heat about 4 c. c. of the prepared solution to boiling, add a few drops of urine and continue boiling for one half minute. If dextrose is present the reddish precipitate of cuprous oxid will appear. If it does not appear, a few drops more of the urine might be added, but even a small amount is usually quickly shown by this test. The solution is prepared as follows:

Dissolve 10.94 g. recrystallized copper sulfate in 83 c. c. distilled water, add 83 c. c. pure glycerol, then add 834 c. c. of a 10 per cent solution of potassium hydroxid. This solution is said to be so delicate that 6 to 8 drops of suspected urine will give a yellow or yellowish red precipitate if sugar is present; and it is claimed that it will keep indefinitely.

Some contend that the addition of the glycerol does not perfectly preserve the solution.

(4) *Boettger's Test.* Render urine strongly alkaline with sodium hydroxid, then add a very small quantity of basic bismuth nitrate (bismuth subnitrate), boil for 2 or 3 minutes. A black precipitate shows the presence of sugar. This precipitate is probably lower oxids of bismuth. In absence of sugar the reagent remains white. Uric acid does not give the reaction with this test; for this reason it is suitable to use it to confirm Fehling's test. Albumen causes the formation of sulfid of bismuth, a black precipitate, hence must be removed.

(5) *Nylander's Test.* To 10 volumes of urine add one volume of Nylander's solution and boil 2 or 3 minutes. The reaction is the same as that in Boettger's test.

Nylander's solution is made by dissolving 10.33 g. sodium hydroxid in 100 c. c. of water; add 2 g. basic bismuth nitrate and 4 g. Rochelle salts; warm and filter.

Fermentation Test. Mix a little yeast with a test tube full of urine, insert a stopper in which is a U tube of which the outer arm extends 2 inches toward the closed end of the tube. See that no air remains in the tube. Invert the test tube and place over night in a beaker in a warm place. If sugar is present it will be changed by fermentation into carbon di-oxid and alcohol. The carbon di-oxid will drive out the urine and its presence can be demonstrated by opening the tube under a solution of sodium hydroxid. This test is of value only in confirmation of other tests.

Quantitative Determination of Sugar.

Measure out in a flask 10 c. c. of Fehling's solution, dilute it with four times its volume of water and heat to boiling. Drop into this hot fluid the urine from a burette one c. c. at a time until the blue color of the Fehling's solution disappears; it may be necessary to let the precipitate settle or to filter off a portion, care being taken to return all the filtrate to the flask. The urine required to decolorize this amount of the diluted Fehling's solution contains approximately 50 m. g. of sugar. Calculate what per cent of sugar the urine solution contains. Dilute some of the urine until it will not contain over .5 per cent of sugar and repeat the determination allowing less of the urine solution to drop into the Fehling's solution at a time, as the point of decoloration is nearly reached. Three determinations which agree closely may be averaged to give a fairly correct estimation of the sugar. The Fehling's solution should be kept hot while the determination is being made.

Laevulose, Lactose, Inosite and Dextrin.

Other sugars such as levulose, lactose, inosite (muscle sugar) and an animal gum termed dextrin are found in urine, but are of little pathological importance and are extremely difficult of detection.

Acetone.—$(CH_3)_2$ CO—may occur in urine in considerable quantities in advanced stages of diabetes mellitus and in fevers. It is believed to be a decomposition product of albumin. Its detection in urine is diffcult and half a liter or more should be distilled after adding a little phosphoric acid. A few cubic centimeters of the first 100 c. c. of the distilled liquid may be used for the following test:

Lieben's Test. To 6 or 8 c. c. in a test tube add a few drops of a solution of iodin in potassium iodid; then add enough sodium hydroxid to make alkaline;—iodoform crystals separate out and may be recognized uuder the microscope by their six-sided plates or star-shaped crystals.

Bile Pigment.

The bile pigments are found in the urine in jaundice and especially when the bile ducts are closed by some obstruction. Certain changes in the blood and hemorrhage into the tissues are followed by the occurrence of biliary pigments in the urine. In cases of phosphorus poisoning this condition of the urine is very marked. When urine contains much bile pigment it is colored yellow, yellowish-brown, brown, greenish-yellow, or greenish-brown.

Tests. Filter some of the urine through a white filter. Upon the filter place a drop of fuming nitric acid; a pale yellow spot will be formed, surrounded by rings of yellowish red, violet, blue and green.

Gmelin's Test. Upon 4 or 5 c. c. of fuming nitric acid in a test tube pour an equal volume of urine so that they will not mix. A band of green will form at the junction of the liquid, due to the biliverdin of the bile. Blue, violet and red rings may also appear, but the green is most characteristic.

A modification of this test is to mix sodium nitrate with the urine and add sulfuric acid to set free the nitrous oxid which gives the characteristic rings with the bile.

Bile Acids.

These occur in urine in so-called bilious attacks, in jaundice, in atrophy of the liver; in increased temperature in fevers, in anaemia, in splenic leucocythaemia and scurvy.

Pettenkoffer's Test.

To recognize the bile acids take 5 c. c. of the urine, add slowly, so that it will flow to the bottom unmixed, half as much concentrated sulfuric acid, then 2 or 3 drops of cane sugar solution and agitate it gently by tapping the tube with the finger until the sugar solution reaches the upper surface of the acid; if bile is present a color which may vary from pink to purple appears. It is sometimes a magenta. Only experience in observing the color will enable one to recognize it. Avoid excess of the sugar solution,

but enough must be used to produce the furfurol necessary to combine with the bile acid to give the color to be recognized.

This test can be relied upon with certainty in highly colored urine, only when the bile acids have been extracted with ordinary alcohol, then with absolute alcohol and after evaporation taken up with water and precipitated as lead salts with lead acetate. They must be again extracted with hot alcohol, filtered, sodium carbonate added, evaporated again and finally taken up with water to be tested as above.

Phosphates.

In normal urine the calcium, magnesium, and sodium salts of phosphoric acid occur. Ordinarily the acid salts are present as NaH_2PO_4, $CaH_4(PO_4)_2$ and $MgH_4(PO_4)_2$ and the acid reaction of the first of these gives to the urine its reaction. When urine is acid all phosphates are held in solution. Upon standing it begins to decompose and becomes alkaline whereupon the calcium and magnesium phosphates are precipitated. Triple phosphate, (ammonium magnesium phosphate) is often found in alkaline urine. If found, in recent urine, it indicates decomposition within the bladder, and is liable to lead to phosphatic calculi.

Phosphates are increased in rickets, meningitis, disorders of the brain, great nervous strain and phosphatic diabetes.

Phosphates are apt to be diminished where there is diminished nutrition; in gout, in any structural disease of the kidneys, as dropsy, in acute diseases, as fevers, and during pregnancy. A diminution of phosphates is regarded as constant a symptom of Bright's Disease as is the presence of albumin itself.

Any single determination may be misleading, so much depends upon the food of the patient. Twenty-four hours urine should be mixed to secure a sample for the determination of phosphates. Excessive or very scanty amount of phosphates indicates some of the above maladies. The amount of phosphoric acid in normal urine varies somewhat. One author gives an average of 2g. in 24 hours; another gives 2.7 g. as an average of many determinations.

Tests For Phosphates.

To some urine in a test tube add a few drops of magnesia mixture; the phosphates will be precipitated in a white cloud. By comparing some normal urine with the suspected, one can soon be able to detect any unusual excess or deficiency.

Magnesia mixture is made by dissolving 100 g. magnesium sulfate and 100 g. of ammonium chlorid in 800 c. c. of water; then add 100 c. c. of strong ammonia water; let stand 24 hours and filter.

The earthy phosphates may be distinguished from the alkaline by adding ammonia and boiling; this precipitates the former. Filter these off and add magnesia mixture which will precipitate the latter.

Determination of Total Phosphoric Acid.

This is made by titrating with uranium nitrate or acetate. If the former is used sodium acetate must be added to the urine to prevent the formation of nitric acid.

Uranium acetate solution is prepared as follows: Dissolve 35 g. of crystallized uranium acetate in a liter of water and standardize against a solution of sodium mon-acid phosphate—10.085 g. pure crystals to one liter of water—until one c. c.of this solution equals 5 m. g. of $P_2 O_5$.

To 5 c. c. of the urine add 50 c. c. of acetic acid, and run into it—drop by drop—the standardized solution of uranium acetate. Place several drops of the potassium ferrocyanid solution on a porcelain plate and from time to time put a drop of the urine upon one of these. When all the phosphates are precipitated the least addition of uranium acetate will cause a reddish brown color with the potassium ferrocyanid. For every c. c. of the solution, there were 5 m. g. of $P_2 O_5$. The amount in 24 hours urine can then be calculated.

Sulfates.

There are two kinds of sulfates present in the urine. (1) The ordinary neutral sulfates of sodium and potassium, and (2) etheral sulfates. In quantity they vary from 1.5 g. to 3 g. per day and are increased or diminished parallel to urea.

Tests.

To a sample of urine add acetic acid and barium chlorid solution; the ordinary sulfates will be precipitated. Filter,and to the filtrate add one c. c. of concentrated hydrochloric acid,then boil a few minutes; this will decompose the ethereal sulfates and they may be precipitated with the barium chlorid already present; if enough is not present, some more may be added.

Determination.

The most satisfactory method of estimation is to precipitate as above, filter, burn filter in platinum crucible and weigh. They may also be titrated with a standard solution of barium chlorid and when no more precipitate forms, the volume of standard solution is measured and the corresponding amount of SO_3 calculated.

Chlorids.

The amount of chlorids in urine varies from 10g. to 15g. with ordinary food. With salt food it may be as high as 25g. daily. Any conclusion based upon variation in amount of chlorids can be reached only by a consideration of the food and habits. There is an increase in diabetes insipidus and temporarily in some fevers.

A decrease may be noted in some febrile disorders. The serous accumulations of pleurisy diminish salt in the urine, and in some cases it nearly ceases. A return of chlorids toward normal amounts would be a favorable indication.

Tests. Silver nitrate solution will form a white precipitate with chlorids,

and its density will enable one to form an idea of scantiness or abundance.

Determination. A standard silver nitrate solution made by dissolving 29.075g. of pure fused silver nitrate in one liter of water and standardizing against sodium chlorid solution. Each c.c. equals 10mg. of sodium chlorid.

Place 10 c.c. of urine, neutral or slightly acid, in an evaporating dish, add about 100 c.c of water and two or three drops of potassium chromate solution. Let the silver nitrate solution run in slowly from a burette; when the chlorids are neutralized, the reddish-brown color of silver chromate appears. If the urine is highly colored 1 c.c. of the standard solution must be deducted before calculating the amount of NaCl. This is called Mohr's method.

Urea. CON_2H_4.

Urea is a normal constituent of the urine and is one of the chief forms in which the nitrogenous parts of the food are excreted. It is excreted in variable quantities, but averages in males from 30 to 40 grams per 24 hours. In women and children the total amount excreted is less, but relatively is more in children.

The amount of urea excreted may be regarded as a measure of the metabolism, where no structural disease of kidneys or liver exists. In fevers it is increased until the decline of the fever begins. It is probable that it is prepared in the liver as acute atrophy or other disease of that organ seems to prevent its formation. Structural degeneration of the kidneys prevents its elimination from the blood. Uraemia is preceded by a decreased elimination of urea and indications of its liability may be had in advance from determinations.

Urea is increased in diabetes; habits of exercise, amount and kind of food and even atmospheric changes may affect the amount of urea excreted, hence caution is necessary in drawing conclusions from analyses.

Tests. The presence of urea in excess may be shown as follows: Place a drop of urine on a glass slide and add a drop of nitric acid, leave in a cool place. If within 5 minutes an abundant crop of crystals of nitrate of urea appear, urea is *in excess.* Evaporate some urine to half its bulk. Take a drop and proceed as in excess. If no crystals are formed in five minutes there is a *deficiency of urea.*

Determination of Urea. The percentage of urea is determined by titrating with a mercuric nitrate solution, (Liebig's method.) Dissolve 77.2g. dry mercuric oxid (or 71.48g. pure mercury) in nitric acid and evaporate to drive off excess of acid, take up in water and dilute to one liter. Prepare a baryta mixture by combining one volume of cold saturated barium nitrate with two volumes of a cold saturated solution of barium hydroxid. Prepare also a solution of sodium carbonate, 53g. to the liter.

To 40 c.c. of the urine add 20 c.c. of the baryta mixture, mix well and filter. Take 15 c.c. of the filtrate in a beaker or flask and pass into it from a burette the murcuric nitrate solution. When the urea is no longer present

a drop of urine containing an excess of the mercuric nitrate will form a yellow color with a drop of the sodic carbonate placed on a porcelain plate. Each c.c. of the mercuric nitrate solution represents 10mg. of urea. If albumen is present it should be coagulated and filtered out.

A deduction of 1g. of urea should be made for every 1.3g. of sodium chlorid in the urine. If one titration reveals more than 2 per cent of urea it must be diluted below 2 per cent.

Doremus' Method. Fill a Doremus apparatus with hypo-bromite solution until tube and bulb are full. Through a curved, graduated pipette introduce 1 c.c. urine solution; nitrogen gas is liberated and rises to the top of the tube, where the reading of its volume indicates the per cent. of urea. Smith's and Squibb's apparatuses are more accurate and depend upon the same principle. They will be shown and the determinations orally described. The hypobromite solution is made by adding 25 c.c. bromin to a solution of 100g. of sodium hydroxid in 250 c.c. of distilled water. The solution does not keep well and the sodium hydroxid solution can be used 10 c.c. at a time and 1 c.c. of bromin added when it is to be used. The bromin should be introduced beneath the surface of the sodium hydroxid solution, and only a little at a time added, as the reaction developes considerable heat.

Uric Acid. $C_5H_4N_4O_3$.

Uric acid like urea is a nitrogenous product. In quantity, compared with urea, it is usually present in about the ratio of 1 to 50. It varies much in quantity. It is supposed to decrease in gout and other diseases where it is found in the blood, but an increase or decrease has little clinical significance, or rather is not well understood. It is not usually present as free acid but combined with the alkali bases as urates. If the urates contain but one atom of the metal they are acid; if both hydrogen atoms of the acid are replaced it is alkaline.

The origin of uric acid is much disputed. There are three theories: (1) That it is formed in the kidneys. (2) That it is formed in the liver. (3) That it results from the decomposition of the nuclei of nucleated cells and is formed wherever the disintegration of these cells goes on.

Tests. Uric acid crystals are easily recognized by their reddish-brown color, their large size and characteristic shapes. Their presence may not indicate an excess of urea but a condition of acidity, and deficiency of other salts in the urine.

Besides the microscopic examination the murexid test may be used. Place the precipitate in an evaporating dish, put a drop of nitric acid on it and evaporate to dryness; a drop of ammonia gives a purple color.

Determination. To 200 c.c. of urine add 10 c.c. of HCl. Allow to stand 48 hours in a cool room. Filter on a weighed filter, wash the precipitate with cold water acidulated with HCl, dry and weigh. Add 0.0048g. for every 100 c.c. of filtrate and wash water. If albumen is present it must be

removed. If urates are not all in solution they must be first dissolved by heating or by sodium hydrolid.

Ehrlich's Diazo-reaction.

An unknown substance found in the urine in typhoid fever, small pox, tubuculosis, typhus and scarlet fever gives what is known as the diazo reaction. When this reaction is first given in tuberculosis it is considered an unfavorable indication and when it ceases in typhoid fever it is considered favorable. It is therefore of prognostic value and may aid in diagnosis.

The solution is made in two parts as follows:

(a) Dissolve 5g. sulfanilic acid in 50 c.c. of HCl and 1000 c.c. of water.

(b) Make a 0.5 per cent. solution of sodium nitrite.

Before using mix one part of b with 50 parts of a. The nitrite solution is liable to oxidation and can not be kept long. To the urine to be tested add equal volume of the mixed reagent and then add an excess of ammonia. A carmine red color which on shakidg reddens the foam, is an evidence of the diazo-reaction. Normal urine gives a red or orange, but not the deep red of this reaction.

SEDIMENT IN URINE.

When fresh urine is allowed to stand for a few minutes a sediment will sometimes begin to settle to the bottom and should be examined by the microscope for pathological constituents. Generally, however, urine is clear and sediment forms only upon longer standing. All urine will decompose, if allowed to remain in a vessel for a day or two.

Collection of Sediment. The sediment may be collected by allowing urine, with two or three drops of chloroform added to prevent decomposition, to stand for a few hours; it may be removed by a pippette from the bottom of the vessel and placed upon a slide.

A better method of obtaining the sediment is to rotate it in a centrifrugal machine at a rate of from 1000 to 1500 revolutions per minute.

Kind of Sediment The sediments usually found in urine are commonly divided into two classes, organized and unorganized.

Organized Sediment. This may consist of blood corpuscles, pus corpuscles, mucus, epithelium, casts of uriniferous tubules, spermatozoa o bacteria

Unorganized Sediment. May be uric acid, urates of the alkali metals, eucin, tyrosin, cystin, cholesterin, fat globules, hippuric acid, calcium carbonate, calcium phosphate, calcium oxalate or magnesium ammonium (triple) phosphate.

Significance and Recognition of Sediments.

Blood Corpuscles.—Significance. The presence of blood in the urine is called hæmaturia, and is always an indication of a pathological condition. When the urine contains *very much* blood, it usually comes from the pelvis of the kidneys, ureters or the bladder, and not from the kidneys themselves.

When the blood comes from the kidneys, as it does in acute Bright's disease, renal calculi, malignant growths in the kidneys, traumatism, etc., the blood is usually nearly equally distributed, or in slender, rod-like clots showing that they have been moulded in passing through the ureters. There is more or less pus with the blood in cases of renal calculi. In hæmaturia of vesical origin the urine is usually alkaline in reaction and phosphates are apt to be present in the sediment. In hæmaturia from the urethra the blood precedes the urine.

Blood Corpuscles.—Identification. Blood usually causes a reddish color, which one may soon learn to recognize. The microscopic examination is the surest and most convenient method for the detection of blood. The corpuscles are bi-concave disks of a yellowish color and with a smooth margin. Upon standing in water or solution of salts the shape is changed. The white corpuscles are larger and have a finely granular appearance. When blood is placed in water the corpuscles may rapidly change shape and have very irregular margins called crenated.

Pus.

The occurrence of pus in the urine is called pyuria. It is found in a wide range of pathological conditions.

When pus originates from the kidneys the urine is apt to retain its normal acidity. Round epithelium and casts may be abundant, and if this is the case the real origin is quite certain. There are several different lesions of the kidneys in which pus may be formed, such as cancer, tuberculosis, nephritic abcess and renal calculi.

When the origin of the pus is in the bladder the urine is apt to be ammoniacal and contain triple phosphates. Any disease of the bladder may occasion it. Calculi, ulcers, tuberculosis and cancer may be mentioned among the possible diseases of this organ.

Suppuration in the ureters is apt to be accompanied by slight colicky pains along their course.

If the pus is in the urethra, it will pass in the first urine and the last of the passage will be free from pus. This occurs in gonorrhœal and other diseased conditions.

An increase, or a considerable quantity maintained for some time, may be a serious indication. The pus corpuscles from deep seated suppurations are apt to be irregular in contour, while in more superficial disorders the pus is normal,

Pus Corpuscles—Identification.

The white corpuscles of the blood, the corpuscles of mucus and the pus corpuscles are identical in structure and form. Their number in blood and mucus in much less and by this means one may distinguish between these three. The test with acetic acid for albumin may show the presence of mucus.

The addition of acetic acid will cause the nuclei of the pus corpuscles to become distinct. There are usually two or three nuclei. Sodium hydroxid added to a sediment of pus will break up the corpuscles and form

a gelatinous fluid the gelatinous character of the fluid may be seen by pouring from one test tube to another. This may be used as a test for pus.

Ephithelium.

Normal urine contains some epithelium and therefore it has a pathological signification only when present in considerable quantities.

The shape of the epithelial cells does not indicate with certainty the portion of the urinary tract from whence they came.

In general, a very large number of the small, round epithelial cells point to the kidneys as the origin. If the cells be mostly of the large, flat kind it may be supposed that the bladder is involved, although the other kinds of cells may come from the deeper epithelial layers of the bladder.

Casts.

Urinary casts are little plugs of proteid matter which are deposited in the tubules of the kidneys and afterward carried out in the urine. They are not composed of precisely the same constituents as any other proteid of the body. They are very certain evidence of the destruction of the tissue of the kidneys. The basis of the proteid matter may enclose other materials giving different appearances to the casts so that they may be divided into several kinds such as:

(1) Those consisting of anatomical elements—blood, pus or epithelium.
(2) Those consisting of products of anatomical substances, waxy, granular or fatty cats.
(3) Those termed "hyaline." These are clean, transparent casts, the nature of which is still a disputed question.

Epithelial casts may be indications of only a temporary desquamation and if they disappear in a few days allow a favorable prognosis. Granular and hyaline casts indicate a graver disease of the parenchyma of the kidney usually becoming chronic. The greater the number and the larger their size the more unfavorable the prognosis. Fatty and waxy casts usually indicate fatty degeneration and are the most serious indication of any.

Casts—Identification.

A complete description of these kinds is not necessary, for the shape is their chief means of identification. They are tubular and their characteristic property is indicated by their name. Care should be taken to become familiar with cotton, linen and silk fibers, so as to avoid confusing them with casts. It sometimes happens that masses of urates or phosphates may lie in shapes similar to casts. The solubility of urates and phosphates will distinguish the one from the other.

Bacteria.

A large variety of bacteria may be found in urine, but their identification would require too much time and would involve a special course in bacteriology. It will be sufficient for our purposes to know that minute, rod-like or spherical bodies, which are to be seen, especially in old urine, are bacteria. Their presence in recent urine indicates decomposition with-

in the bladder. They may be in motion. They are not dissolved in acids as any minute crystals would be.

Unorganized Sediment.

The crystals or amorphous deposits which are frequently found in urine are important indications of changes which are occurring in the metabolism of the body or of changes in the urine before or after its passage.

Uric Acid.

Uric acid occurs in acid urine. It is found most frequently in acute fevers and inflammations. It may occur also in cases of defective action of the liver, or in errors of diet, and is often accompanied by headache, emaciation and hypochondriasis. It frequently occurs in early stages of interstitial nephritis.

It is due (1) to excess of acids, (2) to excess of uric acid, (3) to deficiency of bases, (4) or to deficiency of water. If uric acid crystals are present in urine when passed there is danger of formation of uric acid calculi.

Uric Acid Crystals—Identification.

The crystals of uric acid vary greatly in form, but the most common shape is that of a rhombic prism. They are of yellowish, orange or reddish color and are usually large in size.

Uric acid dissolves readily in alkalis but is insoluble in hydrochloric acid.

Urates of Sodium, Potassium and Ammonium.

Urates are deposited most frequently in fevers, as is uric acid. Diseases of the viscera, liver and functional disorders of the stomach are frequently associated with the deposit of urates in the urine.

Urates—Identification.

The urates may occur in little, spherical masses, or in crystalline forms of star-shaped, needle-like clusters. It is needless to distinguish the bases of the urates, as the clinical significance is in each case the same. The peculiar spherical masses, usually more or less colored, sometimes with spicules projecting from the spherules, are usually recognizable as ammonium urates. Ammonium urate is found in alkaline urine, the potassium and sodium in acid.

By gently heating the urine the urates readily dissolve, but they do not dissolve in weak acids.

Urates and uric acid give the murexid test. To some sediment containing urates in an evaporating dish add a few drops of nitric acid and evaporate to dryness; then add a drop of ammonia and a beautiful purple will appear.

Calcium Oxalate.

Crystals of calcium oxalate appear in the urine in a great variety of conditions. Beneke sums them up as follows:

(1) In impeded metamorphosis in which the oxalic acid is not oxidized into carbonic acid. This may be the case in several forms of illness.

(2) When the nitrogen containing foods are imperfectly changed and more oxalic acid is formed than usual.

(3) Excessive use of proteids, saccharine or starchy foods; insufficiency of red blood corpuscles; insufficient pure air; organic lesions which impede respiration or circulation; depressed conditions of the nervous system.

(4) Excess of alkaline bases in the blood.

Calcium Oxalate Crystals—Identification.

These crystals are usually recognized by their peculiar shape and markings. They appear to be small square plates with light diagonals, or else they resemble minute dumb bells. The former crystals are in reality highly refractive octahedra. Calcium oxalate dissolves in hydrochloric but not in acetic acid.

Phosphates.

Ammonium magnesium phosphates (triple phosphates) are found in urine with ammoniacal reaction; calcium phosphate is found under a similar condition either in amorphous or, less commonly, in crystalline form. They indicate important modifications of the ordinary changes occurring in the blood or the urine. If the reaction is from a fixed alkali, the deposit is chiefly calcium phosphate. The urine is then of a high specific gravity, cloudy, and effervesces with acids. This condition may be temporary and have little significance, or it may be more permanent. General debility, anaemia, defective nutrition, etc., are conditions which are apt to be accompanied by alkaline urine and a deposit of phosphates. These conditions occur after long or exhausting illnes. Flatulent dyspepsia is often a cause of phosphate deposits in urine. There may be excessive elimination of phosphates in nervous irritability, dyspepsia and emaciation. The triple phosphate crystals are chiefly found in ammoniacal urine and are due to cystitis or pyelitis if they occur in the urine when it is voided.

Phosphatic Sediment—Identification.

The triple phosphates occur chiefly in prismatic crystals of which the so-called "coffin lid" crystals are common. These deposits also occur in leaf-like form. Both kinds of crystals are soluble in acetic acid, but not in ammonia.

The calcium phosphate is rarely in crystals, generally in amorphous masses which may be identified by solubility in acetic acid.

Fat Globules—(Lipuria).

In fatty degeneration of the kidneys, in some cases of diabetes mellitus, in chronic parenchymatous nephritis and in phosphorus poisoning, fat globules may be found in the urine.

Fat in the urine is a physiological condition in pregnant women. Oil has been found in urine in heart disease. Lipuria has been reported after administration of cod liver oil. In diseases of the pancreas oil has been noted.

Fat Globules—Identification.

The highly refractive globules have smooth edges and great irregularity in size. They are easily recognized with a little practice.

Fat crystals sometimes appear as bundles of fine needles which disappear upon slightly warming.

Cystin.

Cystinuria has not yet been positively associated with any definite pathological condition. Chlorotic females and scrofulous children seem prone to cystinuria. It has also been found associated with liver diseases. In some families it is apt to form calculi.

Cystin—Identification.

It is found in colorless, six-sided plates, insoluble in boiling water, acetic acid, alcohol and ether. It is distinguished from uric acid or calcium oxalate by its solubility in oxalic or hydrochloric acids; from triple phosphates by its solubility in acetic acid.

Leucin and Tyrosin.

These substances occur together in the urine in phosphorus poisoning, in acute yellow atrophy of the liver and in extensive suppuration, in gangrene, in leucocythæmia, typhoid and small pox.

Leucin and Tyrosin—Identification.

Place the urine to be tested in a watch glass, evaporate one half and allow to cool; leucin and tyrosin, if present, will crystallize out, the former in round masses with fine radiating striæ, the latter in fine bundles of needles.

POISONS.

Introduction.

A poison is somewhat differently defined by writers on the subject, but there seems to be no legal definition.

Any substance which when introduced into the body acts in a noxious manner by means not mechanical, tending to cause death or injury to health, is regarded as a poison.

The introduction into the body may be by absorption either through the pores of the skin, or through the walls of the alimentary tract.

Classification of Poisons.

Poisons may be classified according to their physiological action in various ways; that followed by Draper is simple and practical. He divides them into Corrosive, Irritant and Neurotic.

Corrosive Poisons are those which are destructive of tissue, such as the strong mineral acids, the alkalies and some mineral salts, silver nitrate and corrosive sublimate. Carbolic acid is also a corrosive.

Irritant Poisons are those which produce an inflamed condition of the parts directly affected. Among those may be mentioned arsenic, antimony, phosphorus, cantharides, ergot, copper, mercury, zinc, lead and other compounds.

Neurotic Poisons may be defined as those which, after absorbtion, act on the nervous system and injuriously affect the respiration, circulation or other processes of the body.

Some poisons which belong to the first two classes have neurotic effects also, while the destruction of tissue or irritation produced by the first two may affect the sympathetic system so that grave results follow, unless attention is directed to the condition of the nervous system as well as to the local treatment.

Purposes of Treatment.

Antidotes are used for three purposes: First to get the poisons out of the body, as by vomiting. The stomach pump may be used for this purpose. Second to neutralize or render inactive what may not be removed. Third to combat any dangerous symptoms that may have arisen; by stimulating the free action of the bowles or kidneys; by exciting respiration; by preventing convulsive nervous action or by arousing from the deadly stupor caused by some poisons.

To remove poisons from the stomach by vomiting or stomach pump is dangerous if the lining of the mouth, pharynx and oesophagus is much corroded; and as most of the corrosive poisons may be rendered harmless it is rarely necessary to use emetics or stomach pump in such cases. When

the poison is not highly corrosive, vomiting may be caused by tickling the fauces; or, if that is not successful any of the following emetics may be used: Sulphate of zinc, 20 to 30 grain doses; powdered Ipecac, 20 to 30 grain doses; apomorphine,1-20 1-10 grain doses. Apomorphine may be injected into the blood in 1-60 grain doses. In case none of these are a hand, a teaspoonful of mustard in a cup of warm water may be given; if it does not act promptly the dose may be repeated at intervals of 15 minutes.

Many acids may be rendered inert by the use of any harmless alkali, as magnesia, precipitated chalk, common soda, lime water or even soap suds or wall plaster. Some compounds, however, must be rendered insoluble to save the patient, as the neutral salts may be as poisonous as the compound itself. So that in most cases a knowledge of the reactions of each element is necessary to enable one to remember the proper antidote.

To counteract the effects may be illustrated by the treatment for opium poisoning. The patient is aroused by stimulation; is kept in motion; is excited to exertion in keeping off the dangerous stupor produced by the drug.

For the purpose of better understanding and remembering the treatment, a classification partly chemical and partly physiological is usually followed.

MINERAL ACIDS.

When concentrated, the mineral acids, sulfuric (oil of vitrol,) nitric (aqua fortis,) hydrochloric (muriatic,) corrode the skin and flesh rapidly; destroy cloth or leave red stains; have an intensely sour taste and turn litmus paper red. Dilute acids posess these properties in a less degree.

Tests.

Sulfuric acid gives a white precipitate with barium chlorid solution, insoluble in acids; and with lead nitrate it also gives an insoluble precipitate.

Hydrochloric acid gives with silver nitrate solution a white precipitate insoluble in nitric acid, but soluble in ammonia.

Nitric acid (1) yields brown fumes with pieces of copper foil. (2) If a fresh solution of iron sulfate be poured upon a small amount of nitric acid in a test tube to which one-third its volume of sulfuric acid has been added, a brown ring will form at the surface of the nitric acid. The mixture of nitric and sulfuric acid should be cooled before adding the iron sulfate.

Effects.

These acids attack the mouth, pharynx, esophagus and stomach instantly; and remedies must be immediately administered. One fluid drachm (3½ c. c.) will probably cause death, and even 3 drops of concentrated H_2SO_4 may do so.

Sulfuric acid causes a brown or a black color after a time, though the affected membrane may be white at first.

Hydrochloric acid also causes a white or yellowish appearance.

Nitric acid leaves a yellow stain to the lips and mouth.

Intense pain from mouth to stomach begins immediately and is followed by vomiting. The vomited matters are acid and bloody and contain shreds of mucus. The mucus and blood may be changed to a brownish color by the action of acid. Vomiting may be absent. The mouth and throat are apt to swell so that death may occur from asphyxiation. The bowels are apt to be constipated. The pulse becomes feeble and the skin cold and clammy. Collapse may occur and end in death within a few hours.

Treatment.

Magnesia or chalk suspended in water, baking soda, lime water, soap or wall plaster may be used. With sulfuric acid an abundance of cold water will be beneficial. Soda or chalk produces a gas (CO_2) which distends the stomach, and magnesia is therefore preferable.

The remedy must be given immediately and in sufficient quantities. Lives have been saved by scraping plaster from the wall and administering it with water. Follow the antidote with mucilaginous drinks to soothe the irritation. Flax seed tea, gum arabic in water, or flour paste will serve this purpose. Do not use stomach pump or cause vomiting.

ORGANIC ACIDS.

Acetic, oxalic, hydrocyanic and carbolic acids are the most common organic acids which have caused death by poisoning. Carbolic acid (phenol) is, strictly speaking, not an acid, but is taken up here because of the common use of the name.

ACETIC ACID.

This acid is poisonous only when concentrated and may then be recognized by its odor which is the same as that of vinegar.

Treatment.

For acetic acid the treatment is the same as that for mineral acids.

OXALIC ACID.

Oxalic acid is a white crystalline substance, usually used in solution in water for removing ink stains, cleaning brass, etc. It is sometimes called salts of lemon—a misleading name. It has a sour taste and reddens litmus paper.

Test.

(1) With calcium chlorid solution it forms a white precipitate, soluble in HCl, but insoluble in acetic acid. (2) With H_2SO_4 and heat this acid decomposes into CO and CO_2 which pass off with effervescence; most other organic compounds char when so treated.

Effects.

Oxalic acid and its acid salt of potassium are similar in effects and treatment. They act as corrosive, as irritant and as neurotic poisons,—effects varying according to amounts and dilution. Four grams (60 grains) have caused death; while in other cases more was taken and the patient recovered. The mucous membrane from mouth to stomach is corroded, causing a burning sensation; vomiting is apt to occur, and the vomit may be bloody; pulse becomes slower and finally imperceptible; stupor follows which may end in death. The symptoms vary, but the burning sensations and stupor are frequent. Death may occur in a few minutes, or it may be delayed for days. If the acid is dilute, cramps and numbness are both marked.

Treatment.

Precipitated chalk, lime or magnesia in milk or mucilaginous drinks given freely. Scrapings from wall plaster may be used in emergency. The mucilaginous drinks soothe the irritation. Emetics may be given after a time if no vomiting occurs. Use as little water as possible. The alkaline carbonates should not be used for the alkaline oxalates are as poisonous as the acid itself. They, as well as the acid, affect the nervous system producing stupor or even collapse.

HYDROCYANIC ACID. (PRUSSIC ACID.) HCN.

This acid and potassium cyanid may be considered together. The acid is a volatile liquid. Potassium cyanid is a white solid in powder or lumps. The latter is the more common; used as an insecticide, in manufacturing, etc.

Tests.

The acid gives a peach-blossom odor, is very volatile, reddens litmus feebly, forms white precipitate with silver nitrate, and is soluble in HCl. The salt in solution responds to these tests as it decomposes and forms the acid by standing.

Effects.

It is a neurotic poison and acts very rapidly; one grain (.06 gram) may be fatal. A feeling of constriction in the throat, nausea and vomiting sometimes occur. Giddiness and confusion of sight rapidly set in and the person is apt to fall into convulsions. The mouth may be covered with foam, the eyes glassy and the pupils dilated. Convulsions do not always occur, as some fall lifeless almost at once. The respiration centers are, probably most affected as respiration is peculiar,—the expiration being prolonged and the inspiration shortened. Hydrocyanic acid also affects the blood, preventing absorption of oxygen.

Treatment.

Dash cold water in the face, cause ammonia vapor to be inhaled, artificial respiration. Use stomach pump or emetic as quickly as possible.

CARBOLIC ACID. (PHENOL.) C_6H_5OH.

Though commonly called an acid, phenol is an alcohol; its corrosive action is, however, much like that of strong acids. It is a white, crystalline

substance when pure; but is usually a colorless or brownish liquid owing to impurities. Glycerol is often added to preserve it in liquid form.

Tests.

Its peculiar odor is easily recognized. With dilute ferric-chloride it forms a violet color.

Effects.

Six grams, (90 grains) have caused death. Fifteen grams, (231.5 grains) have proved fatal in a majority of cases. A poisonous dose is followed by burning sensations, pallor with clammy sweat followed by unconsciousness. Vomiting may occur but is not so common as with mineral acids. The pupils are contracted and the breathing noisy and difficult. The poison causes paralysis of the respiratory centers. Death may occur in a few minutes or after 8 or 10 hours. If urine is passed it is usually dark colored. Death with above symptoms may occur from application to the skin and absorption into the circulation; or by strong solutions too freely used as an antiseptic. A five percent solution injected into the vagina after childbirth caused the death of a woman.

Treatment.

Use stomach pump, unless there is too great destruction of mucous membrane, in which case use emetic of 2 grams zinc sulfate (30 grains), or of mustard and water. Oil or mucilaginous drinks may sooth the irritation of the mucous membrane. Prevent collapse by use of stimulants such as aromatic spirits of ammonia,—30 drops to half a glass of water. Brandy is also recommended in such cases by Blythe. In absence of stomach pump or temporarily of emetic, oils or mucilaginous drinks may dilute the poison and delay its absorption, but must soon be removed or serious results may not be avoided.

ALKALIS.

The hydroxids of sodium (caustic soda), potassium (caustic potash), and ammonium, and their carbonates, are usually called alkalis, and are corrosive poisons. The hydroxid of ammonia is a gas usually dissolved in water; the others are white solids. Concentrated lye, common lye and washing soda are alkaline carbonates.

Tests.

The alkalis turn litmus blue, have a soapy feeling and rapidly corrode the epidermis when they come in contact with it. Ammonium compounds yield the odor of ammonia gas when heated with potassium hydroxid. Sodium compounds are recognized by the *yellow* color imparted to a flame; potassium compounds by the *violet* color. It is usually necessary to view the latter with a blue glass as the yellow color imparted by sodium impurities present is apt to conceal the violet colors.

Effects.

The corrosive effects have been already mentioned. Forty grains,

(2.6 grams) of KOH have been fatal. NaOH is less active or energetic, but half an ounce (15 grams) of either would probably be fatal. NH_4OH is even more energetic than either of these.

The ammonia acts upon the respiration besides acting directly upon the tissues. Ammonia expels oxygen from the blood, acts upon hæmoglobin and destroys blood corpuscles. It may affect the air passages and cause death in a very few minutes.

Alkalis produce a burning pain from mouth to stomach, vomiting, and an increase of saliva. The vomited matter frequently contains altered mucus and blood. The tongue and fauces become swollen and the mucous membrane of the mouth and tongue are corroded, leaving a white, spongy surface, easily scraped off. There is frequently a hoarse cough and the swollen condition of the mouth and throat may make breathing difficult. The skin becomes cold and moist. There is usually diarrhœa, accompanied by great pain in the abdomen.

Stricture of the esophagus sometimes occurs long after the patient recovers from the immediate effects of the poison.

The odor of ammonia on the breath, or strong alkaline character of the vomit as shown by litmus paper, or the soapy feeling, would aid in determining the character of the poison.

Treatment.

Do not use the stomach pump or emetic. Give dilute vinegar or lemon juice freely. As in acids, the antidotes must be administered promptly. These should be followed by oil or mucilaginous drinks. Stimulate to prevent collapse, if it is indicated.

MINERAL POISONS.

The mineral salts and oxids of minerals are, many of them, virulent poisons.

ARSENOUS OXID, AS_2O_3.

Arsenous oxid, called arsenic, ratsbane or arsenous anhydrid, is frequently taken by accident or design. It is a white, odorless and nearly tasteless powder. Paris green and London purple are compounds of arsenic as is also Schweinfurth's green. Realgar and orpiment are sulfids of arsenic. A mixture of arsenous iodid and mercuric iodid, known as Donovan's solu tion, is poisonous. Fowler's solution is made of arsenous oxid and potassium carbonate. "Rough on Rats" contains arsenous oxid.

Tests.

Dissolve in HCl, add water and pass H_2S gas through a portion; a yellow precipitate results.

Put another portion in an apparatus for generating hydrogen, which has a jet of hydrogen burning before the solution is introduced; a porcelain dish held in the flame will receive a grayish-black deposit of metallic ar-

senic. If the evolution tube be heated while the gas containing arsenic is being liberated, a dark ring will form. This is known as Marsh's test.

If the tube be taken off and the dark ring heated in a current of air or oxygen a cloud of white oxid of arsenic will form in the cooler part of the the tube and an examination with a magnifying glass will show the beautiful octahedral crystals of arsenous oxid.

Effects.

The smallest fatal dose of arsenous oxid is .13 grams (2 grains). Death has occurred within two hours; it generally occurs in from twelve to thirty-six hours. Arsenic may produce death by application to ulcers or eruptions of any kind, or by injection into the vagina.

The symptoms are those of an irritant and neurotic, and vary much. Blythe recognizes four distinct forms of arsenical poisoning.

Acute Form.—Nausea, vomiting of food and even blood, diarrhœa, coldness of extremities, pulse small, patient sinks in collapse and dies within twenty-four hours.

Sub-acute Form.—Symptoms similar to above but less violent. Scantiness and high color of urine noticeable. Irregular heart action, troubled respiration and cramps in the legs. Death may be delayed several days. This is the most common form.

The Nervous Form.—This form shows paresis, delirium or convulsions while the ordinary vomiting and diarrhœa may be absent.

Chronic Form.

This results from absorption, or small doses continually repeated. The patient may simply be in ill health having loss of appetite, feebleness and an inflamed condition of the nasal passages, as if from a cold. Skin eruption is apt to appear, and hyperaesthesia, or its opposite, paresis, may occur. The symptoms are so varied that they alone will hardly enable one to ascertain their cause. The stomach is, however, almost invariably inflamed.

Treatment.

Freshly prepared hydroxid of iron is the best antidote. The tincture of chloride of iron can be mixed with enough ammonia water to precipitate the reddish-brown hydroxid; filtered or strained by a piece of muslin, the precipitate having been washed with a little water is given freely and as quickly as possible. Cause vomiting soon afterward.

Milk or white of eggs will hinder the absorption and if followed by an emetic will remove a large part of any arsenic which may be in the stomach. This is true, also, of any metallic poisons which is apt to be taken. Allay irritation by the use of oils or mucilaginous drinks.

Compounds of Antimony.

Tartar emetic (potassium antimony tartrate,) and antimony chlorid are the compounds most apt to be met with in practice. The antimony chlorid is a strong corrosive. The wine of antimony is made by dissolving 10 grains (.75 grams) of tartar emetic in an ounce of sherry wine.

Tests.

These compounds dissolve in concentrated HCl, but the dilute acid gives a white precipitate of the basic salts of antimony. Tartar emetic dissolves in water, but not in alcohol. Hydrogen sulphid passed into a pure solution not too strongly acid gives an orange precipitate. Marsh's test shows a black deposit, which with $(H_4N)_2S$ gives an orange color, instead of yellow given by arsenic.

Effects.

Tartar emetic produces nausea, vomiting, purging and pain in the stomach. If a fatal dose is taken, the urine is suppressed, the temperature falls; difficult respiration, spasms and delirium may occur.

Antimony chlorid (butter of antimony) produces inflammation and corrosion of the lining of the alimentary canal. A feeling of nausea accompanied by great pain and burning sensations followed by great prostration results.

Treatment.

Assist vomiting with tepid water, give strong tea freely, or one-half drachm (a teaspoonful) of tannin in warm water. Milk or magnesia and water should be given in addition, when chlorid of antimony has been taken.

Copper Compounds.

Copper sulfate (blue vitriol or blue stone,) and copper sub-acetate, (verdigris,) are the only copper compounds apt to be taken as a poison. The former is blue in color and is usually in large crystals; the latter is greenish and in a powder or small masses.

Tests.

These compounds of copper are soluble in water. If excess of ammonia be added to a solution of a copper salt, a deep blue color results. H_2S gives with a copper salt a black precipitate insoluble in dilute hydrochloric acid.

Effects.

Pain in the epigastrium, vomiting—the vomited matter is apt to be green or blue—and diarrhoea are the first symptoms. Jaundice frequently occurs, if life is sufficiently prolonged. Stupor and coma or cramps and paralysis may precede death. Seven and seven-tenths grams (120 grains) is a fatal dose.

Treatment.

Encourage vomiting by use of warm water, giving milk and white of eggs or baking soda. Allay irritation by oils or mucilaginous drinks.

Lead Compounds.

Lead acetate (sugar of lead,) lead nitrate, and lead carbonate (white lead,) are all poison. The first two compounds are white crystals, soluble in water; the last is commonly used for white paint and is in chalky masses.

Tests.

The acetate and nitrate of lead dissolve in water from which may be precipitated (1) yellow lead iodid by potassium iodid,(2) the yellow chromate by potassium chromate,and [3] a white sulfate by sulfuric acid. The carbonate may be readily dissolved in nitric acid, excess of acid evaporated; and after dissolving in water, tested as above.

Effects.

Acute Poisoning is most common with the acetate, but a large dose is necessary. Twenty-eight grams have been taken without causing death, while several smaller doses are apt to cause severe illness and may prove fatal. The symptoms of acute lead poisoning are a metallic taste in the mouth, burning sensation and dryness of the mouth and throat, and vomiting which usually occurs within fifteen minutes. There is pain in the abdomen and a sense of constriction at the pit of the stomach. Constipation is the rule, but in rare cases purging occurs.

Chronic poisoning may occur among painters by absorption of lead poison from paint, or among those using water conveyed through lead pipes, or in any case where even a small amount of lead is frequently taken into the system. It produces a blue line or spots on the gums, emaciation, pallid complexion, secretions lessened obstinate constipation and colicky pains.

Treatment.

For acute poisoning give Epsom salts (magnesium sulfate), or Glauber's salts (sodium sulfate), followed by an emetic. If no other remedy is at hand milk or white of eggs followed by an emetic will be serviceable. For chronic lead poisoning, remove the source of the poison, keeping the excretory organs active and inducing the patient to drink water freely, is the only treatment that can be recommended.

MERCURY COMPOUNDS.

Mercuric chlorid (corrosive sublimate) and mercurous chlorid (calomel) are the compounds by which mercury poisoning has occurred. The first is a white, crystalline substauce, soluble in water; the second is almost insoluble in water but dissolves sufficiently in the digestive fluids to be to some constitutions poisonous even in few grain doses. Mercurial ointments may cause death from absorption.

Tests.

Soluble compounds of mercury give (1) a black precipitate with H_2S; (2) a bright scarlet with potassium iodid; (3) when acidulated and placed on a copper foil leave a silvery spot. The calomel may be recognized by its insolubility in dilute HCl, and by its black color when moistened with ammonium sulfid.

Effects.

Three grains (.2 grams) of corrosive sublimate have destroyed life; five grains is probably a fatal dose in an average case. Death is apt to occur in from two to six days. This poison is a corrosive and produces intense burning pains from mouth to stomach, nausea, vomiting, diarrhoea with bloody stools, and a swelling of the abdomen. The lips and tongue are white and shrivelled. Death results from collapse. Many smaller doses may produce chronic poisoning resulting in depression, hectic fever symptoms and salivation, often with swollen gums and salivary glands. Calomel is not corrosive but causes salivation and many other symptoms of corrosive sublimate. In large doses it is an irritant poison.

Treatment.

Free use of milk, white of eggs, or flour paste, followed by vomiting may remove the poison in time to save life. In chronic poisoning, the treatment must depend on the symptoms, after the source of the poison has been removed. Potassium iodid is said to destroy compounds made by mercury with the tissues and is therefore used in chronic poisoning.

SILVER COMPOUNDS.

Silver nitrate (lunar causitc) is used for cauterizing, for indelible ink and in photography. It is a powerful corrosive.

Tests.

Soluble in water, it is precipitated by common salt forming the white chlorid which is insoluble in nitric acid, soluble in ammonia and turns dark in the sunlight. It also forms a red precipitate with potassium chromate.

Effects.

Silver nitrate corrodes the mucous membrane, produces vomiting, diarrhœa and convulsions.

Treatment.

Give freely a solution of common salt and produce vomiting. Allay irritation. Although silver nitrate is corrosive, vomiting is necessary as the chlorid is liable to be absorbed in poisonous quantities.

ZINC COMPOUNDS.

Zinc sulfate (white vitriol), and zinc chlorid are soluble salts of zinc which are poisonous. The sulfate is a *mild* irritant, while the chlorid is a *powerful* one. The former is used for an emetic in one scruple (1.25 g.) doses; the latter is a good disinfectant.

Tests.

Dissolved in water (1) ammonium sulfid produces with it a white precipitate, as does also (2) ammonium carbonate and (3) ammonium hydroxid.

Effects.

Both salts produce nausea and vomiting. Great pain in the stomach is caused by zinc sulfate, and great prostration also occurs. Death does not often occur, for the poison is usually vomited out; but when it does, it follows collapse. The chlorid in large doses corrodes the mucous membrane, causing a burning sensation in the mouth and throat, and the vomiting is apt to be accompanied by purging, sometimes with cramps and convulsions.

Treatment.

Give sodium bicarbonate (cooking soda), in water, and milk or white of eggs. Tannin or strong tea will be useful. After any of the above have been freely given, cause vomiting.

COMPOUNDS OF TIN, BISMUTH, CHROMIUM AND IRON.

These compounds are not apt to be taken in poisonous doses, yet they may be.

Treatment.

Magnesia is an antidote for any except bismuth, for which no antidote is known. Vomiting should be used in poisoning from these substances.

POTASSIUM COMPOUNDS.

Potassium nitrate (saltpeter), potassium sulfate and potassium bitartrate (cream of tartar) have caused death, acting as irritant poisons.

Treatment.

No antidote is known. Vomiting must be produced as soon as possible. Demulcent drinks will be found useful to allay the irritation.

BARIUM COMPOUNDS.

Barium compounds are rarely accessible, but the chlorid, acetate and nitrate are poisonous.

Treatment.

Sodium sulfate (Glauber's salts) or magnesium sulfate (epsom salts) should be administered and vomiting produced.

TESTS.

To a solution of stannous chlorid add a few drops of a solution of mercuric chlorid; the stannous chlorid will reduce the mercuric to mercurous chlorid and precipitate calomel. The reduction may be carried so far that some metallic mercury is produced. The precipitate is white or gray.

To another portion of a solution of stannous chlorid add a solution of H_2S and a dark brown precipitate is produced.

To a third portion in which HCl is present, add a few drops of concentrated nitric acid and boil. Divide into two parts and repeat the tests tried above. Explain.

Dissolve a small amount of bismuth sub-nitrate in a little concentrated HCl; add water until a white precipitate appears. It is produced by the change of the $BiCl_3$ to BiOCl by reaction with the H_2O. Dissolve again and add H_2S; note the black precipitate of Bi_2S_3.

To a solution of a chromium compound add silver nitrate; a brownish red precipitate of silver chromate results.

To another portion of the chromium compound in solution add a soluble lead salt; note the formation of a yellow precipitate of lead chromate.

To a solution of an iron salt add ammonium hydroxid; a reddish-brown precipitate of iron hydroxid is formed. Add hydrochloric acid and heat, then add a few drops of nitric acid; with potassium ferrocyanid a deep blue precipitate will be formed.

A potassium compound gives a violet color to a flame; it will also form a yellow precipitate with platinic chlorid.

To a solution of barium chlorid add a few drops of HCl and make the flame test. Note the yellowish-green color of the flame. To another portion add dilute sulfuric acid, a white precipitate of barium sulfate is formed.

PHOSPHORUS.

This is a common ingredient of rat poisons. Many children have been poisoned from sucking the ends of matches. Phosphorus paste is extremely poisonous.

Tests.

Phosphorus is recognized by distilling, after addition of sulphuric acid, and in a dark room when it is shown by its phosphorescence.

Effects.

The immediate effects are vomiting, often of bloody matter, sometimes of matter luminous in the dark. The breath may have the odor of garlic, diarrhœa may be *present* or *absent* and there is great prostration.

These symptoms may pass off and after a day, or even longer, other symptoms develope. The skin becomes yellow (jaundiced), and extravasations occur. The liver becomes enlarged and the urine scanty, containing albumen and often leucin and tyrosin. The symptoms vary considerably—sometimes hemorrhage at other times delirium and convulsions occur. Death may not occur for several days.

Treatment.

There is no satisfactory antidote. Magnesia in water is recommended, with white of egg. French turpentine is supposed to be beneficial. In any case, cause vomiting as soon as possible. *Do not* give oil.

IODIN AND ITS COMPOUNDS.

The tincture of iodin and iodid of potassium have been known to produce death.

Treatment.

Give freely starch paste and water.

GASES.

Chlorin.

This is a yellowish-green gas of suffocating odor and very irritating to the air passages. It is used in chemical experiments and sometimes in the arts.

Carbon Mon-oxid, (stove gas).

This gas is the result of the incomplete combustion of coal, especially in hard coal stoves and charcoal burners. It acts on blood corpuscles.

Carbon Di-oxid (choke damp.)

This gas emanates from caves, wells and mines, is a product of fires and a constituent of the air exhaled by animals. It does not act on blood corpuscles, but excludes oxygen and causes suffocation.

Sulphuretted Hydrogen.

This is a poison which affects the blood, reducing the hæmoglobin. It is found in sewer gas, rotten eggs, and is used in chemical laboratories.

Illuminating Gas.

This gas is a mixture of carbon mon-oxid, marsh gas and other constituents.

Treatment.

Fresh, pure air, dashes of cold water on the face, artificial respiration and, inhalation of ammonia vapor.

VEGETABLE AND ANIMAL IRRITANTS.

Aloes, jalap, croton oil, castor oil seeds, poke berries, wild parsnip, oleander, marsh marigold, cantharides, laburnum and black hellebore are irritants and liable to produce death. Oil of savin, oil of tansy and the leaves or berries of the yew are irritants which are supposed to produce abortion, but more frequently produce irritation of the intestines liable to end in death.

Treatment.

After vomiting, coffee may be useful with mucilaginous drinks to allay irritation. Most of these poisons produce irritation in the intestines and are apt to cause purging; this is especially true of croton oil. The treatment should be such as to relieve the irritation and its effects.

POISONOUS FISH.

There are several kindsof such fish, as conger eel, bladder fish, gray

snapper, and some mussels, which are poisonous. The chief effects are those of simple irritants—vomiting, diarrhœa, depression of the pulse and sometimes a nettle rash.

Treatment.

Emetics, followed by emollients. The diarrhœa can be reached by keeping the bowels open. Emollient enemata and fomentations of the abdomen are recommended by Tanner.

POISONOUS MEAT.

Meat of animals which have died of disease and meats, cheese, etc., which have begun to putrefy have caused serious symptoms and even death. Tbe putrefaction, of flesh causes the formation of ptomaines which are extremely poisonous. Changes in conditions under which putrefaction occurs makes a difference in the compounds formed, which accounts for the fact that far more serious results occur in some cases than in others.

Tyrotoxicon may he formed in ice cream, salads or any food composed chiefly of milk and egg. It is extremely common.

Effects.

These poisons cause nausea, vomiting and diarrhœa. Sometimes there are symptoms similar to those of pneumonia.

Treatment.

Cause vomiting if too great time has not elapsed. Increase the action of the bowels, and counteract the prostration which is apt to result from the poison.

TRICHINOSIS.

Trichinæ are minute, round worms which sometimes infest pork and which, after being eaten by human beings, penetrate to the muscles and encyst. According to Hughes there are three stages:

The intestinal stage is marked by a gastro-intestinal inflammation, with nausea, vomiting and watery diarrhœa, the severity depending upon the number of parasites ingested.

The migration stage shows a typhoid-like fever, rapid, feeble pulse, profuse sweats, intense thirst, dry tongue and lips, red swollen face, with soreness and tenderness of the muscular structure, increased by any muscular act. The mind is apt to be clear but apathetic.

The encapsulation stage. If the number of parasites ingested has been few, recovery may occur in this stage; but if the number has been large, the gastro-enteritis, fever and muscular phenomena are severe. Twenty to fifty per cent. of cases are fatal.

Treatment.

The parasites may be killed by thoroughly cooking the meat. There is little chance of destroying them after they enter the system. After migration has begun nourishing food and quiet may be useful.

Vegetable Neurotic Poisons.

The gummy substance called opium is obtained from the unripe pods of the poppy, but its poisonous properties are chiefly due to an alkaloid called morphine. Godfrey's powders, Dover's powders, laudanum, paregoric and many soothing syrups, Dalby's carminative, and other preparations, contain the same substance. Some people, and especially young children, are very susceptible to its action and a small amount may produce symptoms of poisoning.

Effects.

Giddiness, drowsiness and stupor are caused by a large dose. The patient sinks into a state resembling that of a sleep. The breathing becomes slow and noisy, and the pulse, which was full at first, becomes weak. The pupils become contracted, but may expand before death. Vomiting sometimes occurs, but frequently it is not followed by the results hoped. The bowels are usually constipated. In the earlier stages the patient may be aroused by loud noises or shaking, which fact differentiates opium poisoning from epilepsy.

Treatment.

Remove the poison from the stomach by stomach pump or vomiting. Stimulate by dashes of cold water, mechanically, and by strong coffee. Electricity has been recommended. Fresh air in abundance is necessary and the patient must be as thoroughly roused as possible.

Chloroform and Ether.

These are colorless and volatile liquids which produce insensibility by inhalation. They are apt to produce slight stimulation at first, excitement and incoherence and finally complete insensibility.

Treatment.

Fresh air, dashes of cold water and artificial respiration.

Alcohol.

This is too well known to need description. In many cases it produces acute poisoning, and forms of chronic effects are numerous.

Effects.

In acute poisoning the person becomes insensible, the face usually flushed and the pupils dilated. Care should be taken to distinguished between intoxication and apoplexy or concussion of the brain. The smell of alcohol on the breath should not be regarded as sufficient evidence that the case is one of intoxication only.

Treatment.

Apply cold water to the head after removing the poison from the stomach. Coffee or aromatic spirits of ammonia are usually administered internally.

Nitro-benzene, Aniline and Camphor.

These may produce an intoxicating effect and should be treated as alcoholic intoxication is.

Mushrooms.

These may produce the symptoms of pure intoxication, or they may produce effects similar to gastro intestinal catarrh and a disordered condition of the nervous system. There is no good rule by which one may detect poisonous mushrooms; one must be familiar with the species.

Atropin (*daturin*,) $C_{17}H_{23}NO_3$.

This is an alkaloid found in Atropa belladonna and contained in the preparation given as a medicine called belladonna. It is also found in Datura stramonium [Jimson weed,] and Datura alba, a plant growing in India. The seeds of the last named are sometimes mistaken for those of red pepper and have been frequently administered with food to enable the natives to rob a traveler.

Effects.

Very small doses may be fatal and in a very short time. The first sensation is usually a dryness in the throat. The muscles of the pharynx are apt to contract spasmodically. Nausea and vomiting may occur and the pupils are much dilated. Vision is indistinct or double. The skin is dry and may be covered with a scarlet rash. There is usually delirium followed by stupor. The action of the heart is affected, the terminations of the vagus in the heart muscles being first excited, then paralyzed. The delirium in adults is frequently manifested by a disposition to talk much and to see objects at which the patient picks. The active delirium may pass into coma and end in death.

Treatment.

The stomach pump or emetics, castor oil and animal charcoal, are recommended by Tanner. Five drops of laudanum every quarter of an hour is recommended by Walker. Blythe would inject one-fifth of a grain of pilocarpin from time to time. The laudanum may counteract the delirium, but its use is to be guarded.

Hyoscyamus [*henbane*].

This is similar in its action, in many respects, to atropin. It produces giddiness and dilates the pupils, but it produces perspiration instead of dryness of the skin. Delirium is absent if vomiting occurs. It is less apt to be fatal than atropin.

Treatment.

Stomach pump or emetics and increased action of the bowels.

Solanin [*nightshade.*]

This is similar in its action to the poison last described. It is obtained from the berries or leaves of "bitter-sweet" and the "nightshade." The treatment is the same as for hyoscyamus.

Cocaine.

This is obtained from Erythroxylon coca, a shrub of South America. It is much used as a local anaesthetic and as a medicine.

Effects.

Five grains taken internally may produce symptoms of poisoning. It affects the heart and lungs, the pulse becoming rapid and intermittent, respiration slow and feeble. It is classed as a deliriant and the treatment is the same as for the other deliriants.

Nux Vomica.

This, and the alkaloids which are obtained from it, *strychnin* and *brucin* are neurotic poisons producing convulsions. The action on the spinal cord causes the convulsions. The brain is unaffected, as the patient may be conscious until death. Strychnin is more frequently used in medicine and in poison for vermin, hence is most liable to be taken accidentally or with suicidal intent in poisonous doses. The convulsions, or a highly excitable condition of the nervous system is always present. There is a stiffness about the neck and jerking movements of the muscles; a sense of suffocation and difficulty of breathing. The patient is apt to be conscious of approaching convulsions and call out to those about him. As death approaches the tetanic spasms closely succeed each other until the patient is no longer able to survive them.

Treatment.

Remove the poison as in case of other neurotic poisons and quiet the convulsions by laudanum. The greatest care should be taken to avoid any noise, quick motions, or anything to excite the patient. Chloroform may be used to counteract the convulsions, care being taken not to administer too much. If convulsions have begun it may be impossible to use the stomach pump.

Curare, the Calabar Bean and Conium (hemlock).

These are poisons which cause paralysis of the motor nerves. Curare paralyzes the sensory nerves first; the others, the motor nerves only. The hemlock (conium maculatum), is the only one found in the United States. The calabar bean grows in Africa; curare is from a plant which grows in South America and was used by the natives to poison arrow heads. It is more poisonous when introduced directly into the blood than when taken directly into the stomach. In the latter case it is excreted with sugar in the urine. The calabar bean contracts the pupil of the eye.

Treatment.

After removing the poison from the stomach, resort to artificial respiration, as death is caused by paralysis of the muscles of respiration and of the heart. Few cases of poisoning have occurred; but some have been kept alive by artificial respiration until the effect of the poison disappeared sufficiently to allow respiration to go on unaided. Some give hypodermically, one-sixtieth grain doses of atropin to counteract the effects of the calabar bean; others give chloral in ten grain doses.

Aconite.

This is a very deadly poison, one-fiftieth of a grain having caused ser-

ious illness. Aconitin is the alkaloidal base. Several plants native to Europe and Asia contain the poison.

Effects.

Heat, numbness and tingling in the mouth are noticeable. Giddiness and loss of muscular power are marked symptoms of aconite poisoning The pupils dilate, the skin becomes cold and the heart's action is impeded. It resembles curare in that the nerve terminations, rather than the centers, are paralyzed. The sufferer may remain conscious, loss of action of involuntary muscles may follow, causing syncope which may end in death.

Treatment.

Remove poison by stomach pump or vomiting. Give castor oil or charcoal. Strong coffee may be useful. Keep up the action of the heart and lungs by artificial respiration.

Gelsemium.

This poison, containing the alkaloid gelsemine, is a motor depressant, affecting the spinal cord. Poisonous doses produce vertigo, double vision, dilated pupils, slow and feeble heart, labored respiration, anaesthesia, diaphoresis and staggering gait. Death is caused by the paralysis of the muscles of respiration.

Treatment.

Remove the poison from the stomach, stimulate the action of the heart and muscles of respiration by artificial means.

Digitalis, Nicotine, Colchicum and Veratrum (white hellebore.)

These poisons are all heart depressants. Of these, digitalis is more frequently used and is more apt to be taken accidentally or by design. It causes vomiting, purging, headache, *great slowness and irregularity of pulse*, great weakness, convulsions, coma and death. The symptoms vary considerably, but its action on the heart is always present.

Treatment.

For digitalis give emetics and tannin or strong tea. Strong coffee or other stimulants may be needed to lessen depression. The treatment for others named in this connection is similar, except that castor oil may be used instead of tannin.

Rattlesnake Bite.

The bites of poisonous reptiles such as rattle-snakes, water moccasin, and others, cause paralysis by action on the centers of respiration.

Treatment.

Suck the poison from the wound immediately. The poison is not dangerous if accidently swallowed, as it is destroyed by the digestive fluids. Aromatic spirits of ammonia (2 drams to one and one-half ounces of water) or brandy are usually given to stimulate respiration until the poison is eliminated. Potassium iodid in 20 grain doses is perhaps the best remedy. For stings and bites of insects ammonia is the best remedy; but soap, soda or any alkaline substance, gives relief, as the irritant is usually an acid.

SOLUTIONS FOR URINALYSIS AND PHYSIOLOGICAL CHEMISTRY.

Acid, Sulfuric, (concentrated.)—Commercial; or chemically pure is sometimes required.

Acid, Sulfuric, (dilute)—One part of above to 4 parts of distilled water.

Acid, Nitric, (con,)—Strong commercial, unless c. p. is required.

Acid, Nitric, (dilute)—One part of above to 4 parts of distilled water.

Acid, Hydrochloric, (con.)—The c. p. is usually required, commercial may sometimes be used.

Acid, Hydrochloric, (dilute)—One part of above with 4 parts distilled water.

Acid, Acetic.—One part of glacial with 4 parts of distilled water.

Ammonium Hydroxid, (ammonia water)—One part strong to 3 parts water. Should be free from CO_2 gas.

Ammonium Carbonate.—One part powdered crystals in 5 parts dilute ammonia water.

Ammonium Chlorid,—One part pure crystals in ten parts water.

Ammonium Molybdate,—Three g. crystals in 20 c.c. water pour into 20 c.c. of strong nitric acid. Warm to 40°, (not above) and let settle. Make in small quantities; it does not keep well.

Ammonium Oxalate,—One part of crystals dissolved in 20 parts water.

Ammonium Sulphid,—Dilute strong ammonia water with equal volume of distilled water. Saturate 3-5 of this mixture with H_2S, add remainder of mixture and stir.

Barium Chlorid,—One part of crystals to ten parts water.

Calcium Hydroxid,—Slake pure lime and add large excess of water. Pour off water after it settles and again add pure water; shake well, let settle and draw off clear liquid into bottle which must be kept tightly stoppered.

Lead Acetate, basic,—170 g. lead acetate dissolve in 800 c.c. boiling, distilled water. Add 120 g. yellow lead oxid and boil for half an hour, keeping up water to same volume. Let cool and add previously boiled, cold water to 1000 c.c. Filter in covered funnel; keep well stoppered.

Magnesia Mixture,—Dissolve 100 g. magnesium sulfate and 100 g. ammonium chlorid in 800 c.c. water, and add 100 c.c. strong ammonia water. Let stand 24 hours and filter.

Mercuric Chlorid,—One part pure crystals to 30 parts water.

Millon's reagent,—Dissolve 1 part of mercury in 2 parts strong nitric acid, finally by aid of heat; cool, dilute solution with twice its volume of water.

Potassium Bichromate,—One past crystals to ten parts water.

Potassium Chromate,—One part crystals to ten parts water.

Potassium Ferrocyanid,—One part crystals to 20 parts water. For quantitative estimation of albumen 1 to 10.

Potassium Hydroxid,—One part sticks to ten parts water.

Sodium Hydroxid,—One part of sticks (purified by alcohol) to ten parts water. Paraffine stoppers of bottle to prevent sticking.

Sodium Hypo-chlorite,—75 g. chlorid of lime, rub to thin cream with 200 c.c. water. Let settle and pour clear liquid on filter. Wash with 100 c.c. water, allowing entire liquid to flow into same vessel. Dissolve 150 g. sodium carbonate crystals in 300 c.c. water and pour into the other solution. Warm and stir well. Filter, and pour through the filter enough water to bring to 1000 c.c. Keep in dark.

SOME SPECIAL SOLUTIONS.

Fehling's Solution.

(1) Dissolve 69.28 g. pure copper sulfate in distilled water and make up to one liter. Label "Fehling's No. 1."

(2) Dissolve 100 g. sodium hydroxid sticks in 500 c.c. water; heat to boiling and add 150 g. recrystallized Rochelle salts. Stir until dissolved, let stand 24 hours, filter through asbestos and make up to one liter.

The sodium hydroxid should be of the grade precipitated by alcohol. Label "Fehling's No. 2." These two mixed in exactly equal parts make Fehling's Solution. Do not mix until ready to use.

Nylander's Solution.

This solution is made by dissolving 10.33 g. sodium hydroxid in 100 c.c. of water; add 2g. basic bismuth nitrate and 4g. Rochelle salts; warm and filter.

Tanret's Mercuric-Potassium Iodid Solution.

Dissolve 33.12 g. pure potassium iodid in 200 c.c. distilled water. Add 13.54 g. powdered mercuric chlorid; warm and stir until red precipitate of mercuric iodid disappears leaving a clear, yellowish solution. Dilute with distilled water to sbout 800 c.c. and add 100 c.c. of pure, strong acetic acid. Allow to stand over night if not clear, and decant any precipitate that it may contain. Dilute then to one liter with distilled water. This solution contains the proportion of 4KI to $HgCl_2$.

Esbach's Solution.

This solution is made by dissolving 10 g. of pure picric acid and 20 g. of pure citric acid in a liter of distilled water. This solution must be filtered, if it is not perfectly clear.

Haines' Solution.

Dissolve 10.94 g. recrystallized copper sulfate in 83 c.c. distilled water, add 83 c.c. pure glycerine, then add 834 c.c. of a ten per cent solution of potassium hydroxid. This solution is said to be so delicate that 6 or 8

drops of suspected urine will give a yellow or yellowish red precipitate if sugar is present; and it is claimed that it will keep indefinitely. To make the test, heat about 4 c.c. of the solution, add a few drops of the urine and boil one-half minute. Red cuprous oxid is precipitated. Some contend that the addition of the glycerine does not perfectly preserve the solution

APPARATUS AND ANTIDOTES FOR THE TREATMENT OF POISONS.

[Recommended by Blythe.]

1. Stomach pump.
2. Emetics:

Sulfate of zinc, 20 grain (1. 3 gram) tablets.

Ipecacuanha, 20 grain (1. 3 gram) tablets.

Mustard, one-half ounce (16.6 grams) to one pint (496 c. c.) water, thoroughly mixed.

When vomiting cannot be produced by the above owing to the condition of the stomach, an injection by hypodermic syringe of a few drops of a two-per cent solution of APOMORPHIN HYDROCHLORATE will cause vomiting in a few minutes.

3. Antidotes:

1 Acetic acid or vinegar for alkalis.
2 Calcined magnesia for acids.
3 Tannin for alkaloids or antimony.
4 Chloral or chloroform for convulsants.
5 Aromatic spirits of ammonia as a stimulant.
6 Extract of coffee as a stimulant.
7 Dialyzed iron. The tincture of iron may be precipitated with ammonia and filtered for preparation of reddish precipitate of ferric hydroxid for arsenical poisoning, instead of dialyzed iron.
8 French oil of turpentine for phosphorus.
9 Castor oil as a demulcent.
10 Brandy may be required in cased where danger of collapse exists. It should always be remembered that white of egg, flour in water, milk, flax seed tea or other mucilaginous drinks may be useful in a large number of cases, and probably will never do any harm.

EQUIVALENT WEIGHTS AND MEASURES.

1 meter=39.37 inches.
1 liter=33.815 U. S. fluid ounces.
1 cubic centimeter=16.221 U. S. minims.
1 kilogram=32.151 U. S. Apoth. ounces.
1 kilogram=35.274 avoirdupois ounces.
1 gram=15.432 grains.
1 U. S. fluid ounce=29.57 c.c.
1 U. S. Apoth. ounce=31.104 g.
1 avoirdupois ounce=28.349 g.
1 grain=64.798 mg.
1 U. S. fluid ounce weighs 0.95 U. S. Apoth. ounce.

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